INFLUENCE OF INPUT FROM THE FOREWING STRETCH RECEPTORS ON MOTONEURONES IN FLYING LOCUSTS

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Summary

1. Previous studies on the forewing stretch receptors (FSRs) of locusts have suggested that feedback from these receptors during flight contributes to the excitation of depressor motoneurones and reduces the duration of depolarizations in elevator motoneurones. We have investigated these proposals by measuring the timing of FSR activity relative to depressor activity and by examining the effects of stimulating the FSRs on the membrane potential oscillations in flight motoneurones.

2. Activity in the FSRs was recorded in tethered intact animals flying in a windstream and in preparations that allowed intracellular recordings from motoneurones during flight activity. The timing of FSR activity was similar in both preparations. In most animals we observed that at normal wingbeat frequencies (about 20 Hz) the activity in the FSRs commenced *after* the onset of activity in the wing depressor muscles. As wingbeat frequency declined there was a progressive advance of FSR activity relative to depressor activity. Most of the spikes in each burst of FSR activity occurred during the time that the membrane potential in depressor motoneurones was repolarizing.

3. Electrical stimulation of the FSRs timed to follow the onset of depressor activity slowed the rate of repolarization, decreased the peak hyperpolarization and increased the rate of the following depolarization in depressor motoneurones. In elevator motoneurones, the same pattern of FSR stimulation produced an additional excitatory input during the depolarization phase and, at low wingbeat frequencies, reduced the duration of the peak depolarizations. The reduction in the duration of the peak depolarization in elevator motoneurones was not strongly correlated to the reduction in cycle period.

4. We propose that the primary reason why input from the FSRs increases wingbeat frequency is because this input reduces the degree of hyperpolarization in depressor neurones and thus promotes an earlier onset of the next depolarization in these neurones.

Introduction

The forewing stretch receptors (FSRs) in the locust are important propriocep-

tors in the generation of the motor pattern for flight. One indication of their importance is that their removal slows the wingbeat frequency significantly (Wilson and Gettrup, 1963; Kutsch, 1974). The FSRs discharge phasically near the peak of wing elevation (Möhl, 1985*b*), they make excitatory monosynaptic connections to depressor motoneurones (Burrows, 1975) and to a variety of elevator and depressor interneurones (Reye and Pearson, 1987), and electrical stimulation of their axons promotes the onset of depressor activity (Möhl, 1985*c*) and shortens the duration of depolarizations in elevator motoneurones (Wolf and Pearson, 1988). These observations have suggested that the FSRs have at least two separate functions: (1) to contribute to the activation of depressor motoneurones and by so doing limit the amplitude of wing elevation (Burrows, 1976), and (2) to reduce the durations of the depolarizations in elevator motoneurones and thus increase the wingbeat frequency (Wolf and Pearson, 1988). The main aim of this investigation was to evaluate these suggestions by measuring the timing of FSR activity relative to depressor activity and by examining the influence of activity in the FSRs on the membrane potential oscillations in depressor and elevator motoneurones.

Little is known about the influence of activity in the FSRs on depressor motoneurones. The notion that they contribute to burst generation in these motoneurones has been inferred from the observations that they are excited by wing elevation and that they make direct excitatory connections to depressor motoneurones (Burrows, 1976; Wendler, 1983). There are, however, some indications that the FSRs may not normally contribute to the activation of depressor motoneurones. For example, some of the records published by Möhl show that the FSR activity often commences after depressor activity (Möhl, 1985*b,c*; Möhl and Neumann, 1983), and deafferentation has no obvious effect on depressor depolarizations (Wolf and Pearson, 1987*a*). In the present study we have re-examined the timing of FSR input relative to depressor activity and consistently found that input from the FSR occurs too late to provide an additional excitatory input during the depolarization phase in depressor motoneurones. We conclude that the main action of the FSRs on the depressor motoneurones occurs during the hyperpolarization phase. This conclusion was supported by our observations on the effects of electrically stimulating the FSRs on the hyperpolarization phase in the depressor motoneurones.

In the absence of input from the forewing stretch receptors the depolarizations in elevator motoneurones consist of two distinct components: an early component, initiated by phasic input from the hindwing tegulae, and a late component, generated when the wingbeat frequency is below about 16 Hz. Electrical stimulation of the FSR afferents suppresses the late component, shortens the duration of the depolarization in elevator motoneurones and increases the wingbeat frequency (Wolf and Pearson, 1988). Although these observations have suggested that the suppression of the late depolarization is the mechanism by which input from the FSRs maintains high wingbeat frequencies (Wolf and Pearson, 1988), this has not yet been proved. By electrically stimulating the FSRs to mimic their

activity in flying animals we have confirmed that they can suppress a late depolarization in elevator motoneurones, but a quantitative analysis of this phenomenon revealed that the magnitude of the suppression is not strongly correlated to the decrease in cycle period. Thus, we conclude that the suppression of the late depolarization is not the primary mechanism by which input from the FSRs maintains a high wingbeat frequency. Instead, we propose that the way in which the FSRs increase the wingbeat frequency is by antagonizing the hyper-polarization phase in depressor neurones (interneurones and motoneurones) thus allowing an earlier and more rapid onset of the subsequent depolarization.

Materials and methods
Animals

Male and female *Locusta migratoria* were obtained from a colony at the University of Alberta.

Preparations

The first preparation we used was designed to allow recording of forewing stretch receptor activity under conditions as natural as possible. Following the removal of a small flap of cuticle above one mesothoracic nerve 1, bipolar hook electrodes (50 μm diameter copper wires insulated except for the ends) were placed on the nerve. The electrodes were supported temporarily by waxing them to a manipulator. The exposed nerve and the electrodes were then lifted clear of the haemolymph and embedded in dental moulding material (Reprosil Light Body, De Trey). The fine wires of the electrodes were attached to a small rod waxed to the ventral cuticle. The electrodes were then detached from the manipulator. Electromyographic (EMG) recording electrodes (50 μm diameter insulated copper wires) were also implanted in the ipsilateral forewing first basalar muscle to monitor flight activity (see Pearson and Wolf, 1987, for site). The wires from these electrodes were waxed to the ventral cuticle. Following the placement of the electrodes the animal was mounted on a thin rod attached to the pronotum and put in a device that allowed free movements around the yaw axis and then placed in an upright position in front of a wind tunnel. Flight sequences lasting for up to 2 min were initiated by a continuous windstream directed towards the head. The animal established its own final orientation with respect to the wind by rotating about the yaw axis. Signals from the recording electrodes were amplified and stored on magnetic tape for further analysis (see below).

The second preparation we used allowed recording from, and stimulation of, one FSR while simultaneously recording intracellularly from flight motoneurones. As previously described by Wolf and Pearson (1988), animals were mounted ventral side up so that their wings could move freely. The cuticle above the meso- and/or metathoracic ganglia was removed. Bipolar recording electrodes were placed on either prothoracic nerve 6 or mesothoracic nerve 1 to monitor the activity of one FSR. The nerve on these electrodes was insulated using silicone

grease. EMG recording electrodes were placed in the ipsilateral forewing first basalar muscle. A rigid stainless-steel plate was placed under either the meso- thoracic or the metathoracic ganglion (depending on which ganglion intracellular recordings were to be taken from) and the ganglion was further stabilized by placing another rigid plate on top of the ganglion (Wolf and Pearson, 1987*b*). At all times the ganglion was kept covered by saline. Flight was induced either by blowing briefly on the animal or by directing a constant windstream towards the head.

The effect of selectively stimulating the FSRs on the oscillations in membrane potential in flight motoneurones was examined in the second preparation. The stretch receptor afferents were stimulated by monopolar electrodes placed on the two prothoracic nerves 6 (see Pearson *et al.* 1983). To eliminate signals from the forewing stretch receptors due to wing elevation the two mesothoracic nerves 1 were cut distal to their point of joining the prothoracic nerves 6.

Intracellular recordings were made from the first basalar and tergosternal motoneurones in either the meso- or metathoracic ganglion. The somata of these motoneurones in each hemiganglion are large and usually visible. Penetration of the forewing first basalar motoneurone was confirmed by the 1:1 correspondence of spikes in the recording with spikes in the EMG taken from the corresponding first basalar muscle (see Fig. 5). Microelectrodes were filled with $1\,\mathrm{mol\,l^{-1}}$ potassium acetate. Normally these electrodes had resistances of about $20\,\mathrm{M\Omega}$.

Analysis of the timing of stretch receptor activity

The objective of this analysis was to determine the time of onset of FSR activity relative to the onset of activity in the ipsilateral first basalar muscle (M97). Recordings from mesothoracic nerve 1 were first high-pass filtered to define more clearly the stretch receptor spikes from spikes in other afferents and from the axon of a dorsal longitudinal motoneurone in nerve 1 (see Fig. 1). The filtered record was then passed through a window discriminator (WPI model 121) to produce digital pulses corresponding to each spike in the stretch receptor (see Fig. 1). The first spike in each burst recorded from M97 was also digitized by passing the EMG through a monostable circuit that rejected all but the first spike. The digitized pulses were then fed to a general purpose computing facility (DEC LSI 11/23) that was programmed to determine the time of occurrence of each stretch receptor spike relative to the first spike in M97. The program either sorted the data according to cycle period (see Figs 2A, 4A) or plotted the onset time of stretch receptor activity *versus* the cycle period (see Figs 2B, 4B).

Analysis of the effects of electrically stimulating FSRs

The objective of this analysis was to determine how input from the FSRs influenced the membrane potential oscillations in flight motoneurones. This was done by first storing the section of the intracellular record of interest in a Nicolet 4094C digital oscilloscope. This instrument allowed the stored record to be expanded and shifted along the time axis and any section of the stored record to be

plotted. Thus, different sections of the stored record could be superimposed in repeated plots (HP ColorPro printer). Normally the cycle immediately preceding the stimulus train was compared with the cycle occurring coincident with the stimulus train (depressors) or immediately after the stimulus train (elevators). Cursors on the oscilloscope allowed accurate measurements of the amplitudes of the potentials and interspike intervals.

Results

Timing of forewing stretch receptor (FSR) activity

The timing of FSR activity relative to the onset of activity in the forewing depressor muscles has not been analyzed in detail. Examination of raw data in the publication by Möhl (1985b) shows that there can be considerable variation in the timing of the onset of FSR activity. For example, Fig. 2 in Möhl's report shows that the onset of activity occurs earlier relative to the onset of hindwing depressor (M127) activity as the cycle period increases. At high wingbeat frequencies the onset of FSR activity occurs after hindwing depressor activity and therefore could not have been contributing directly to the activation of these motoneurones. At lower wingbeat frequencies the onset of FSR activity precedes hindwing depressor activity. The initial aim of this investigation was to obtain more quantitative data on the timing of FSR activity relative to the activity in wing depressor muscles.

Fig. 1 shows recordings taken from the mesothoracic nerve 1 and the ipsilateral forewing first basalar muscle (M97) in an animal flying in a windstream. M97 was chosen as reference in this analysis for two reasons: the motoneurone supplying this muscle receives the largest monosynaptic excitatory input from the forewing stretch receptor (Reye and Pearson, 1987; Burrows, 1975) and it is activated late in the depressor phase of the flight cycle (Möhl, 1985a). Therefore this motoneurone is more likely than other depressors to be influenced by input from the FSRs. The data in Fig. 1 are similar to those presented by Möhl (1985b) with regard both to the number of spikes per cycle and to the frequency of the stretch receptor activity (in Möhl's report recordings were made from N1D2). In this short sequence the onset of stretch receptor activity preceded the onset of activity in M97 but lagged behind the onset of activity in the dorsal longitudinal motoneurone whose axon projects in N1 [large spike in N1 (filter)]. A quantitative analysis of the timing of the onset of FSR activity relative to the activity in M97 showed, in all animals ($N=4$), that the interval depended on the wingbeat frequency. One example is shown in Fig. 2. Data in Fig. 2 were obtained by analyzing 340 consecutive cycles in a single flight episode. The timing of each spike in the stretch receptor burst was measured relative to the onset of activity in M97 and displayed as a raster plot (Fig. 2A) in which the data were ordered according to cycle period (short cycle periods at the top progressing to long cycle periods at bottom). Three features were apparent in these raster plots: (1) at high wingbeat frequencies the FSR activity usually commenced *after* the onset of activity in M97 (the vertical line in Fig. 2A indicates the time of onset of activity in M97), (2) most of the spikes in the

FSR occurred within 15 ms following the onset of depressor activity, and (3) as the cycle period increased the FSR activity advanced relative to depressor activity. At longer cycle periods (>60 ms) the onset of FSR activity always preceded the onset of activity in M97. This dependence on cycle period is illustrated in Fig. 2B. Despite considerable scatter it can be seen that the delay between the onset of
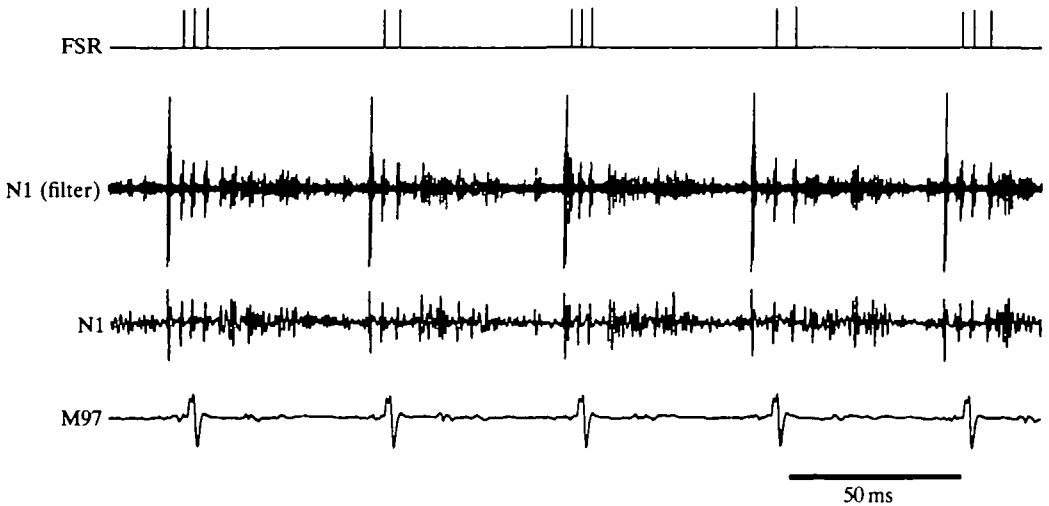

50 ms

Fig. 1. Activity of a forewing stretch receptor (FSR) in a tethered flying locust. Bipolar hook electrodes were placed on a mesothoracic nerve 1 (N1) and implanted into the animal. Flight activity was monitored by electromyogram (EMG) electrodes placed in the ipsilateral forewing first basalar muscle (M97). The nerve recording (N1) shows activity in a dorsal longitudinal motor axon (large spike preceding the spike in M97), the FSR (spikes immediately following the motor axon spike) and other afferents (mostly from the tegula). To extract the spikes from the FSR from the nerve 1 recording, the signal was first high-pass filtered [N1 (filter)]. Filtering exaggerated the potentials from large axons because the extracellularly recorded spikes from large axons have higher frequency components (Stein and Pearson, 1971). The filtered signal clearly shows the distinctions between the motor spike, the FSR spikes and the spikes from other afferents. The filtered signal was passed through a window discriminator set to detect the FSR spikes. The output from the discriminator is shown in the top trace (FSR).

Fig. 2. Timing of FSR activity in a tethered flying animal relative to the onset of activity in the ipsilateral forewing first basalar motoneurone (M97). Data from 340 cycles in a single flight episode. (A) The time of occurrence of the spikes in the FSR (dots) was plotted relative to the time of the first spikes in M97 (vertical line). The 340 cycles were arranged according to cycle period (short periods at the top progressing to long periods at the bottom). The curved line on the left indicates the cycle period. Note that as the cycle period increased the time of onset of the FSR activity advanced relative to the onset of activity in M97. Note also that most of the spikes in the FSR occurred after the onset of activity in M97. (B) Scatterplot showing that the delay between the onset of activity in M97 and the first spike in each FSR burst was dependent on the cycle period. Note that as the cycle period increased the delay progressively decreased.

activity in M97 and the first spike in the FSR burst decreased as cycle period increased.

Similar results to those shown in Figs 1 and 2 for tethered flying animals were
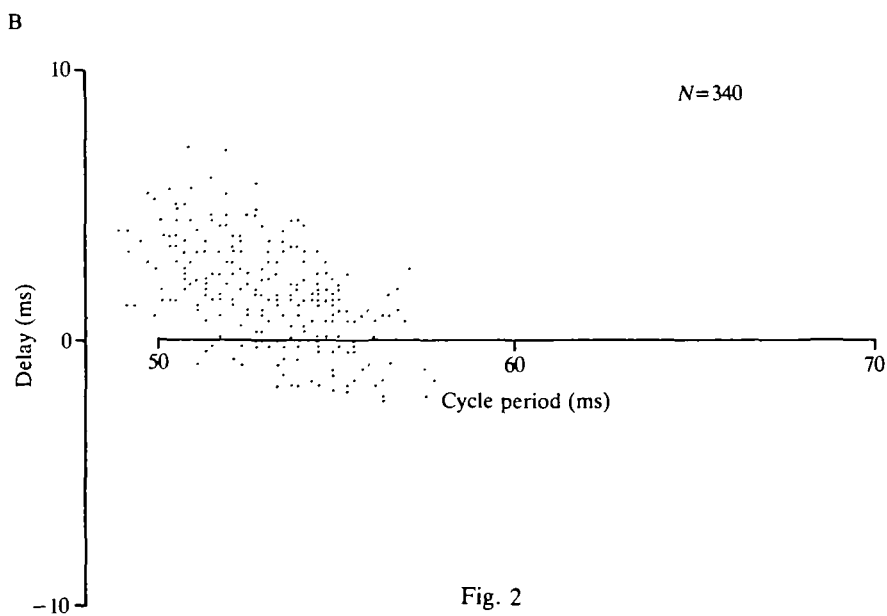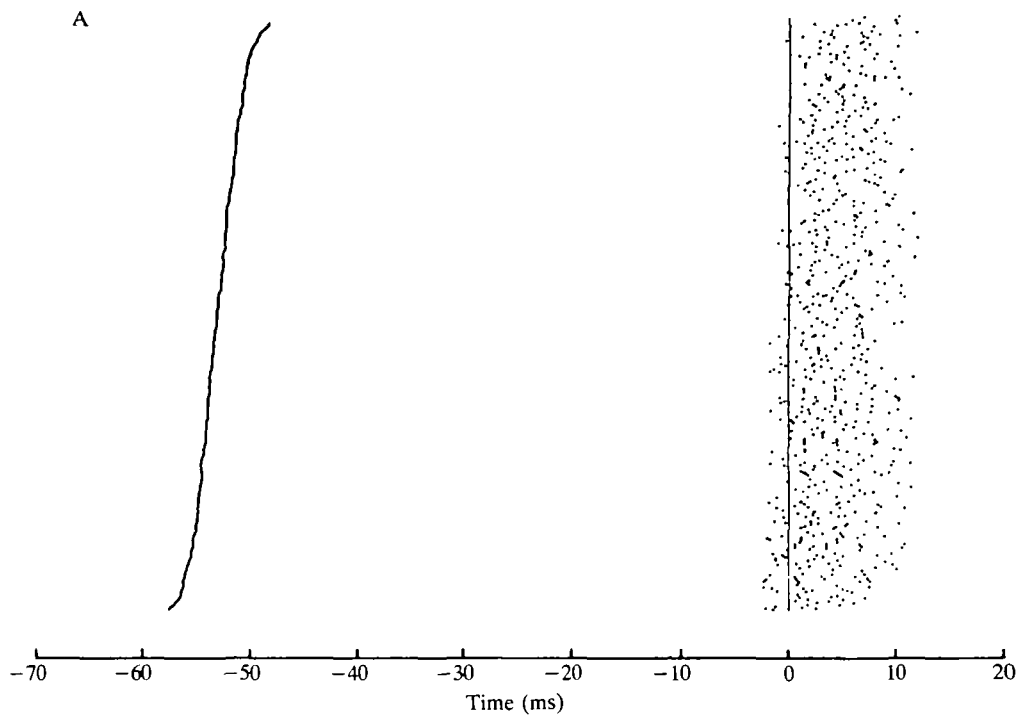
A

B

N=340

Fig. 2

Fig. 3. Recordings of activity in a FSR in a restrained flying locust that allowed intracellular recording from flight motoneurones. The activity in the FSR was recorded from its axon in prothoracic nerve 6 (N6, the nerve was crushed close to the prothoracic ganglion to eliminate spikes in the dorsal longitudinal motor axons). Flight activity was monitored by recording the EMG in the ipsilateral forewing first basalar muscle (M97). At high wingbeat frequencies (A) the onset of FSR activity occurred after the onset of activity in M97. At lower wingbeat frequencies (B) the onset of FSR activity advanced relative to activity in M97, so that its onset occurred before the onset of activity in M97. The records in A and B were taken from the same flight sequence.

also obtained in more restrained preparations designed to allow intracellular recordings from flight motoneurones (Figs 3, 4). Fig. 3 shows two short periods taken from a long sequence in one of these preparations. At high wingbeat frequencies (approx. 20 Hz) the FSR activity occurred after the onset of activity in M97 (Fig. 3A), while at low wingbeat frequencies (approx. 16 Hz) the onset of FSR activity preceded activity in M97 (Fig. 3B). Fig. 4 displays the results of a quantitative analysis of a long flight sequence (200 cycles) in another preparation. This sequence was characterized by a substantial decline of the wingbeat frequency, thus providing a clearer illustration of the dependence of the timing of FSR activity on cycle period. The raster plot (Fig. 4A) shows a progressive shift of the FSR burst (dots) relative to the onset of the activity in M97 (vertical line) as the

Fig. 4. Timing of FSR activity in a restrained flying locust relative to the onset of activity in the ipsilateral forewing first basalar muscle (M97). Data from 200 cycles in a single flight sequence. (A) The time of occurrence of the FSR spikes (dots) was plotted relative to the time of the first spike in M97 (vertical line). The 200 cycles were arranged according to cycle period (short periods at the top progressing to long periods at the bottom). The curved line on the left indicates the cycle period. Note that as the cycle period increased the time of onset of FSR activity advanced relative to the onset of activity in M97. (B) Scatterplot showing the dependence of the delay between the first spike in M97 and the first FSR spike on cycle period.
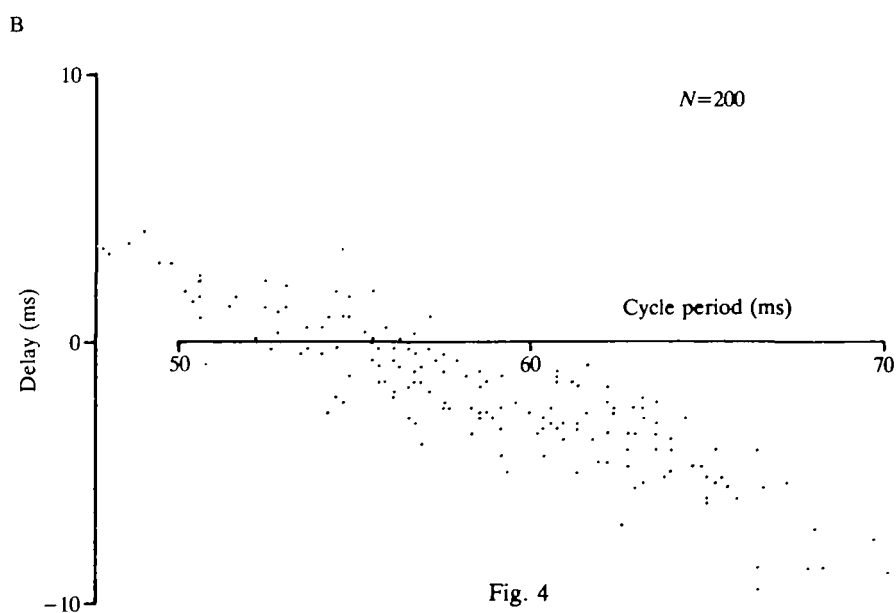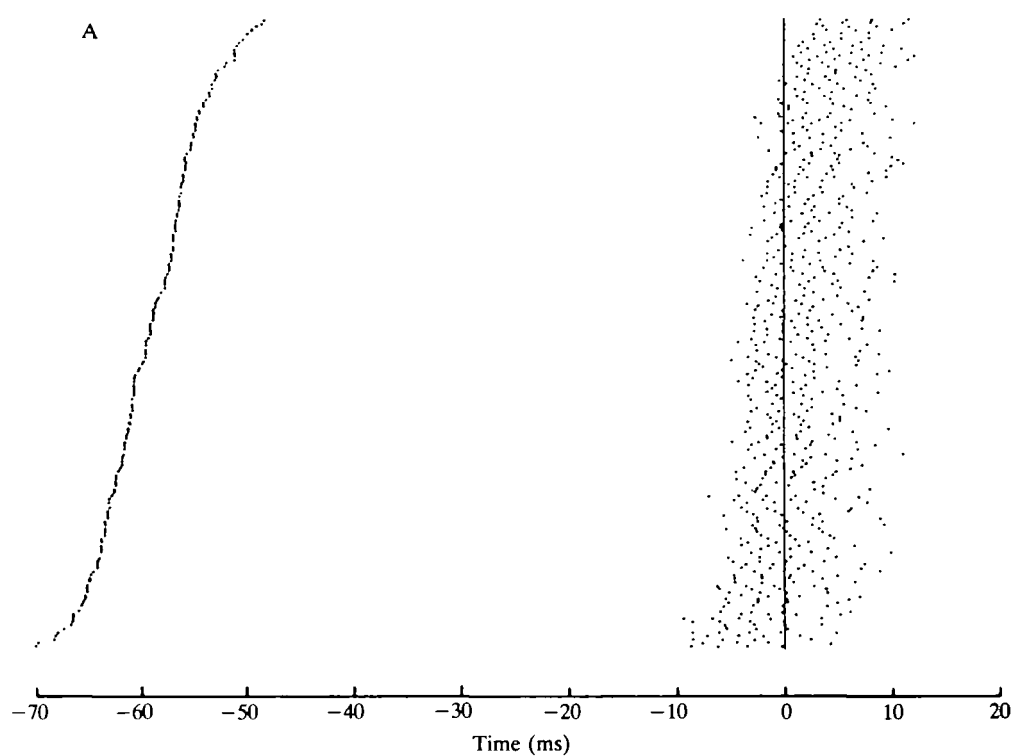
A

Time (ms)

B

N=200

Delay (ms)

Cycle period (ms)

Fig. 4

cycle period increased from about 50 ms to almost 70 ms. The delay in the onset of
activity in the FSR relative to the first spike in each depressor burst is shown in
Fig. 4B. This scatterplot clearly illustrates the dependence of the onset of FSR
activity relative to the onset of activity in M97. This dependence was observed in
all restrained preparations ($N=10$), as was the fact that at high wingbeat
frequencies the FSR activity commenced a few milliseconds after the onset of
depressor activity.

Two conclusions can be drawn from the data presented in Figs 1–4. The first is
simply that the timing of FSR activity is similar in restrained and in tethered flying
animals. This is important because the data obtained from intracellular recordings
(see below) will very probably reflect the events that normally occur in flying
animals. The second is that input from the FSRs is not normally involved in
activating depressor motoneurones, since the onset of FSR activity follows the
onset of depressor activity by a few milliseconds.

Effect of FSRs on synaptic input to depressor motoneurones

Our conclusion that input from the FSRs occurs too late to contribute to the
activation of the depressor motoneurones was confirmed by recording intracellu-
larly from forewing first basalar motoneurones in tethered flying animals. Fig. 5A
shows an intracellular recording from a first basalar motoneurone and the EMG
record from the muscle innervated by this motoneurone. The main points to be
noted are that there is a delay of approximately 3 ms between a spike in the
motoneurone and the corresponding EMG spike, and that the beginning of the
fast rising phase of the depolarization (arrowhead) occurred approximately 10 ms
before the first EMG spike. Thus, for the FSRs to influence the rapid excitation of
the motoneurone, the onset of FSR activity would have to precede the first EMG
spike in M97 by between 3 and 10 ms plus the time for the FSR to conduct from the
recording site and generate an EPSP, which we estimate to be at least 5 ms (see
Fig. 7A). In none of our preparations (tethered or restrained) did this occur
(Figs 1–4). When recordings were made simultaneously from a first basalar
motoneurone and the ipsilateral FSR (Fig. 5B) our most common observation was
that all the spikes occurred during the hyperpolarization phase (Fig. 5C).
Occasionally the first one or two spikes in an FSR burst were timed near the peak
depolarization (particularly at lower wingbeat frequencies), but because of the
time required to generate EPSPs (see Fig. 7) we conclude that it is unlikely that
these spikes contribute to the excitation of the first basalar motoneurone.

Further evidence that input from the FSRs acts on the depressor motoneurones
during their hyperpolarization phase came from a study of the effects of
electrically stimulating the FSRs on the membrane potential oscillations in these
motoneurones. Input from the FSRs was eliminated by transecting mesothoracic
N1s distal to their junction with the prothoracic N6s, and both FSRs were
selectivity activated by electrically stimulating the branch projecting in each
prothoracic N6. Stimulus trains were delivered simultaneously to both FSRs, each
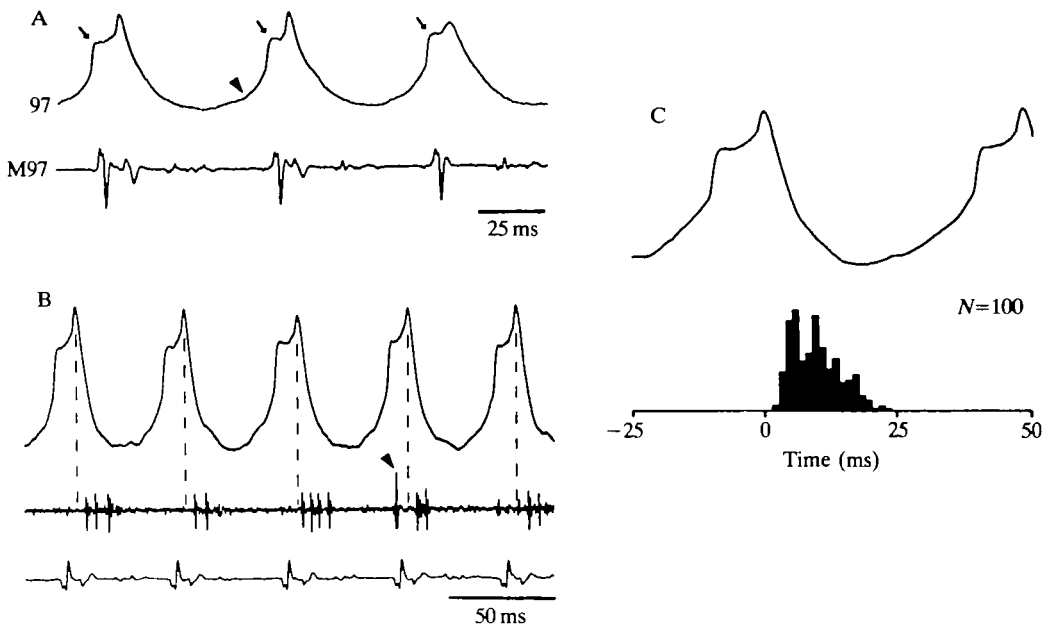train being triggered by spike activity in a forewing first basalar muscle (M97).

Fig. 5. (A) Intracellular recordings from the soma of the forewing first basalar motoneurone (97) in restrained flying animals showing the timing of the oscillations in membrane potential relative to spikes recorded in the muscle innervated by the motoneurone (M97). Note that the spike in the motoneurone preceded the muscle spikes by approximately 3 ms. The arrowhead indicates the beginning of the rapid depolarization leading to spike generation. The arrows indicate the first spike in each depressor burst. (B) Simultaneous recordings of intracellular activity in a forewing first basalar motoneurone (top), spike activity in the ipsilateral FSR as recorded from mesothoracic nerve 1 (middle) and EMG activity in the basalar muscle innervated by the motoneurone (bottom). The large spike (arrowhead) is from the dorsal longitudinal motor axon in nerve 1. The dashed lines indicate the peaks of the depolarizations in the motoneurone. Note that the onset of FSR activity occurred during the time the motoneurone was repolarizing. (C) Histogram showing the time of the occurrence of spikes in the FSR relative to the beginning of the repolarization phase in the ipsilateral forewing first basalar motoneurone. The representative intracellular trace above the histogram allows the timing of FSR activity relative to the membrane potential oscillations to be visualized. Note that in this preparation all activity in the FSR occurred during the repolarization phase. Data are from 100 cycles in a single flight sequence.

Thus, the simulated pattern of activity in the FSRs was similar to that in intact animals. Previous studies have shown that selectively stimulating the FSRs in this manner increases the wingbeat frequency (Pearson *et al.* 1983; Reye and Pearson, 1988).

Fig. 6A shows an example of the effects of FSR stimulation on the profile of synaptic input to a hindwing first basalar motoneurone. Stimulus trains began midway through the sequence. Two effects of the stimulation can be seen. The first was an immediate shortening of the cycle period (by about 8 ms) and the second
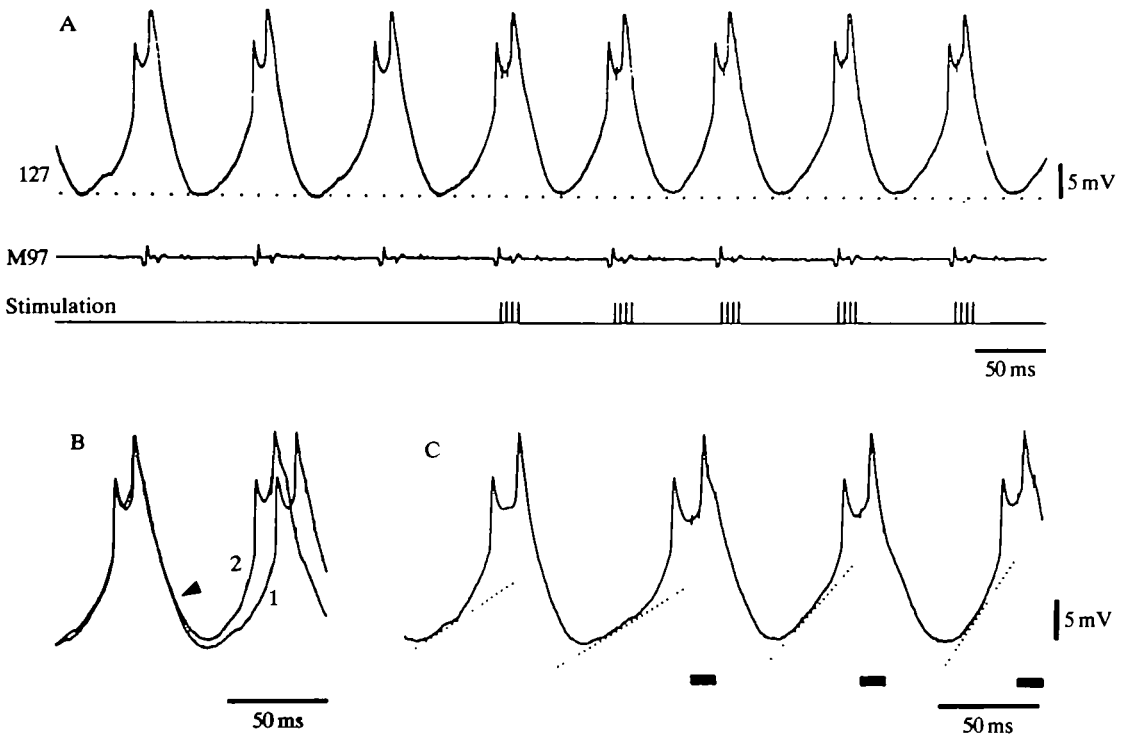
Fig. 6. Electrical stimulation of the FSRs opposes the repolarization phase in depressor motoneurones. (A) Intracellular recording from a hindwing first basalar motoneurone (127) showing that phasic stimulation of the FSRs caused a decrease in the cycle period. The stimulus trains (4 pulses at 250 Hz) were triggered from the large spikes in the EMG from M97. Note that FSR stimulation also caused a slight reduction in the peak negativity of each oscillation (the mean value before stimulation is indicated by the dotted line), and that the rate of depolarization increased. (B) Superposition of a cycle immediately before the first stimulus train (trace 1) and the cycle occurring coincident with the first stimulus train (trace 2) showed that the FSR stimulation slowed the repolarization phase (onset indicated by arrowhead), reduced the peak negativity and increased the rate of depolarization of the next cycle. (C) Sequence of activity showing that FSR stimulation increased the rate of the initial depolarization in motoneurone 127. The dotted lines indicate the slopes of the initial depolarizations and the thick bars show the time of the stimulus trains. Data in A, B and C are from different sequences in the same animal.

was a more rapid depolarization following each hyperpolarization. The latter effect is shown more clearly for another sequence in Fig. 6C. Closer examination of the effects of FSR input showed that this input also influenced the repolarization of the cycle in which it was first delivered and reduced the peak negativity of the hyperpolarization. The superimposed records in Fig. 6B show the hyperpolarization phase of the cycle immediately before the delivery of the first stimulus train (trace 1) and the cycle coincident with the stimulus train (trace 2). Two consistent effects were observed on all trials. The first was that the repolarization was slowed,

beginning approximately 12 ms after the first impulse in the stimulus train, and the second was that the magnitude of the hyperpolarization was reduced by between 0.5 and 1 mV, as recorded from the soma. A quantitative analysis of the change in the magnitude of the hyperpolarization in one animal showed that the reduction was 0.68 ± 0.27 mV. This value was significantly less (Student's t-test, $N=19$) than the difference in magnitude of the two cycles immediately preceding the first stimulus train $(0.02\pm0.32$ mV).

What pathways are involved in mediating the FSR effects on the hyperpolarization phase? One pathway that is probably involved is the monosynaptic excitatory pathway from the FSRs to the depressor motoneurones (Burrows, 1975; Reye and Pearson, 1987). Obviously any activity in the FSR that occurs just before or during the hyperpolarization phase will, *via* this monosynaptic pathway, reduce the level of hyperpolarization in depressor motoneurones. Evidence for a stronger polysynaptic pathway to depressor motoneurones was obtained by stimulating the FSRs in quiescent animals (Fig. 7). Single stimulus pulses delivered to one FSR gave small, short-latency EPSPs in ipsilateral depressor motoneurones (Fig. 7A) and no response in contralateral depressor motoneurones. This was expected from previous studies on the connections of the FSRs (Burrows, 1975; Reye and Pearson, 1988). However, when the FSRs were stimulated repetitively with high-
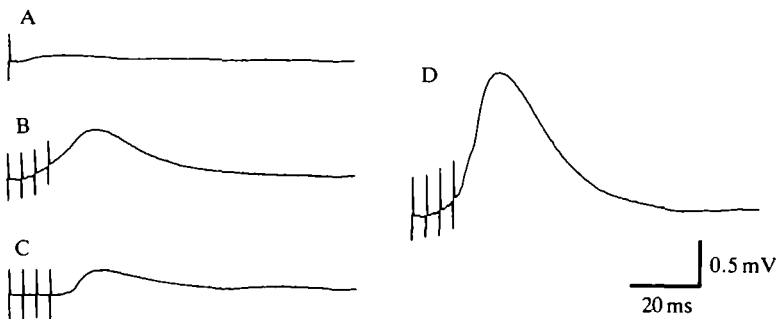

Fig. 7. Repetitive stimulation of the FSRs evokes a delayed EPSP in depressor motoneurones. Intracellular recordings from hindwing first basalar motoneurone showing the synaptic potentials evoked by electrical stimuli delivered to the ipsilateral FSR (A,B), contralateral FSR (C) and to both FSRs (D). The FSRs were stimulated *via* their branch in prothoracic nerve 6. The stimulus artefacts show the time of stimulus presentation. Each trace is the average of 30 stimulus presentations. (A) A single impulse in the ipsilateral FSR evoked a small short-latency EPSP in the motoneurone. This EPSP is mediated *via* the monosynaptic pathway from the FSR to depressor motoneurones. (B) Repetitive stimulation $(250\,\mathrm{s}^{-1})$ of the ipsilateral FSR evoked a large EPSP, the amplitude of which exceeded the estimated sum of the monosynaptic EPSPs. (C) Repetitive stimulation of the contralateral FSR evoked a long-latency EPSP. Note that this EPSP was not preceded by a depolarization, since the depressors do not receive a monosynaptic connection from the contralateral FSR. (D) Simultaneous activation of both FSRs evoked a large EPSP, the amplitude of which was greater than the sum of the EPSPs evoked by each FSR alone.

frequency trains a larger EPSP was evoked in both ipsilateral and contralateral depressors (Fig. 7B,C), and simultaneous activation of both FSRs evoked a much larger compound EPSP in depressor motoneurones (Fig. 7D). The latency of these compound EPSPs was about 15 ms, with the peak occurring at about 25 ms. Since the onset of FSR activity usually precedes the peak hyperpolarization in depressor motoneurones by 20 ms or more, it is probable that activity in this polysynaptic pathway also contributes to reducing the level of hyperpolarization in depressor motoneurones. It must be remembered, however, that transmission in either the monosynaptic and/or the polysynaptic pathway could be modified in flying animals.

Effect of FSRs on synaptic input to elevator motoneurones

In a previous study it was shown that input from the FSRs can suppress the generation of a late depolarization in elevator motoneurones when the wingbeat frequency falls below about 16 Hz (Wolf and Pearson, 1988). It was proposed that suppression of this late component and the associated reduction in the duration of the elevator depolarization may be the reason why input from the FSRs increases the frequency of the flight rhythm. The mechanisms by which the FSRs could suppress the late component of the elevator depolarization remained unresolved. Specifically, the problem was to explain the long latency between the onset of FSR input and the time the late component was suppressed (approx. 25 ms).

To investigate the effects of the FSRs on the elevator motoneurones we electrically stimulated both FSRs via their branch in prothoracic nerve 6 following transection of the mesothoracic nerves 1 distal to their junction with prothoracic nerves 6. The onset of the stimulus trains occurred a few seconds after the initiation of the flight by a wind stimulus to the head. Fig. 8A illustrates the effect of input from the FSRs on the depolarization in an elevator motoneurone. The most obvious effect was a reduction in the duration of the depolarizations (similar to that described by Wolf and Pearson, 1988) and a shortening of the cycle period. Comparison of the elevator depolarizations before and after the onset of FSR stimulation showed that the depolarizations were not equally reduced in duration over their entire height. Instead, FSR input had a greater influence in reducing the duration of the depolarizations near their peak (marked as c in Fig. 8A) than near their base (marked as b in Fig. 8A). The larger effect of FSR input on the duration of the peak depolarization resulted in the appearance of a distinct plateau in repolarization. This plateau was an exaggeration of an inflection that was often apparent in the repolarization phase before FSR stimulation (see second cycle in Fig. 8A). Since the reduction in the duration of the peak depolarization was completed about 5 ms before the final repolarization of the elevator moto-neurones, it follows that this phenomenon cannot be the primary reason for the shortening of the cycle period. Instead, the process that produces the final repolarization from the plateau appears to be associated with the reduction of the cycle period. This conclusion was supported by an analysis of the relationships between the durations of the peak and base depolarizations and cycle period
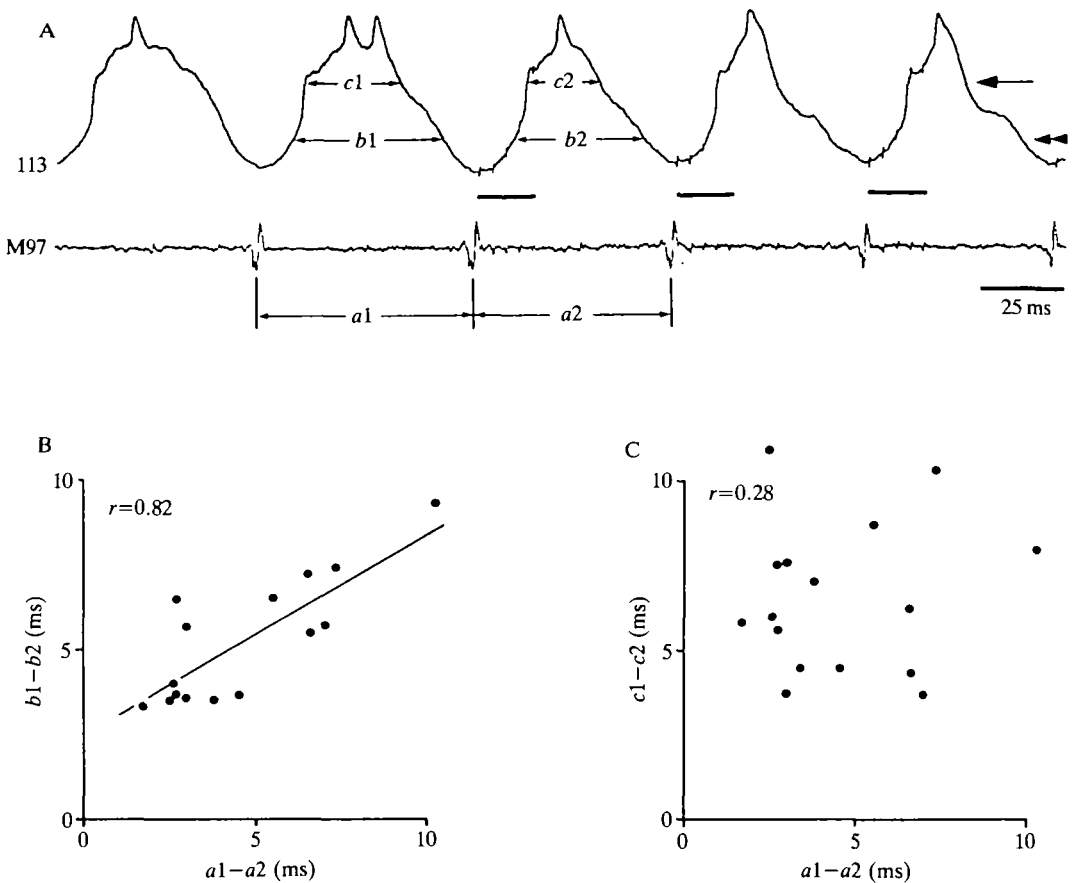
Fig. 8. Effect of electrical stimulation of the FSRs on the oscillations in membrane potential in an elevator motoneurone (113). (A) Trains of stimuli (horizontal bars, 5 pulses at $300\,s^{-1}$) were delivered midway through a flight sequence. This caused an immediate reduction in cycle period and a reduction in the duration of the depolarizations in the motoneurone. The effect of the stimulus trains on the peaks of the depolarizations (c, horizontal arrow) differed from their effect on the bases of the depolarizations (b, double arrowhead). After three stimulus trains this resulted in the appearance of a distinct plateau in the repolarization phase. The difference in the effect of FSR activity on the peak and base depolarizations was also apparent in a quantitative analysis of the effects of the first stimulus train. (B,C) Relationships between the reduction in the duration of the base depolarization ($b1-b2$ in A) and peak depolarization ($c1-c2$ in A) and the reduction in the cycle period ($a1-a2$ in A). Note that the reduction in the base depolarization was more strongly correlated with the reduction in cycle period. Data for these plots were obtained from 15 flight sequences in the same animal.

(Fig. 8B,C). This analysis revealed a poor correlation between the reduction in the duration of peak depolarization and the reduction in cycle period (Fig. 8C). In contrast, the reduction in the duration of the base of the depolarizations (at levels below the inflection or plateau) was strongly correlated to the reduction of cycle

period (Fig. 8B). Thus, we conclude that the process that reduces the cycle period is associated with the events responsible for producing the sudden late repolarization following the plateau.

We also observed that the FSRs contributed an excitatory component to the initial depolarization in elevator motoneurones (Fig. 9). This effect produced a significant reduction in the interval between the first two spikes in the elevator burst (Fig. 9B). Occasionally a fairly distinct, additional excitatory input was observed superimposed on the peak depolarization (Fig. 9A), but this was not always apparent, i.e. a reduction in the interspike interval often occurred without a noticeable effect on the peak depolarization. We regard the effect on the interspike interval to be a more sensitive measure of excitatory input from the FSRs, since some components of excitatory drive to the elevator motoneurones may not always be visible in recordings from the somata. From those cases in which additional synaptic input was visible it was possible to estimate the latency of the excitatory input to elevators from the FSRs. This was within the range 15–20 ms (Fig. 9A), which corresponds closely with the latency of delayed EPSPs evoked in elevator motoneurones when the FSRs are stimulated in quiescent animals (see Fig. 2 in Reye and Pearson, 1987).

Discussion

Timing of FSR activity

One of the main aims of this study was to establish the timing of the input of FSR activity relative to the onset of activity in the forewing depressor motoneurones. The specific question we wished to answer was: is the timing of FSR activity appropriate for the FSRs to contribute to the activation of depressor motoneurones? Two main features of the timing of FSR activity should be noted. The first is that, as cycle period increased, the activity in the FSRs advanced relative to the activity in the forewing first basalar muscle (M97) (Figs 2, 4). The second is that, at normal wingbeat frequencies (approx. 20 Hz), the onset of FSR activity usually occurred *after* the onset of activity in M97. Intracellular recordings have shown that the rapid initial depolarization in the first basalar muscle occurs 3–10 ms before the first EMG spike in the muscle (Fig. 5A). When allowance is made for conduction time and the time for generating the EPSP (approx. 5 ms, Fig. 7A), the FSR activity as recorded from mesothoracic nerve 1 would have to precede the spike in M97 by 8 ms or more for it to contribute to the activation of depressor motoneurones. We *never* observed this in any tethered or restrained preparation flying with wingbeat frequencies around 20 Hz. Thus, at high wingbeat frequencies, the FSRs could not have contributed to the activation of forewing depressor motoneurones. Nor could they have contributed to the activation of the hindwing depressor motoneurones, since hindwing depressor activity precedes forewing depressor activity by approximately 7 ms. Even at lower wingbeat frequencies it is unlikely that the FSRs contributed to the activation of wing depressor motoneurones, because the delay between the onset of FSR activity and
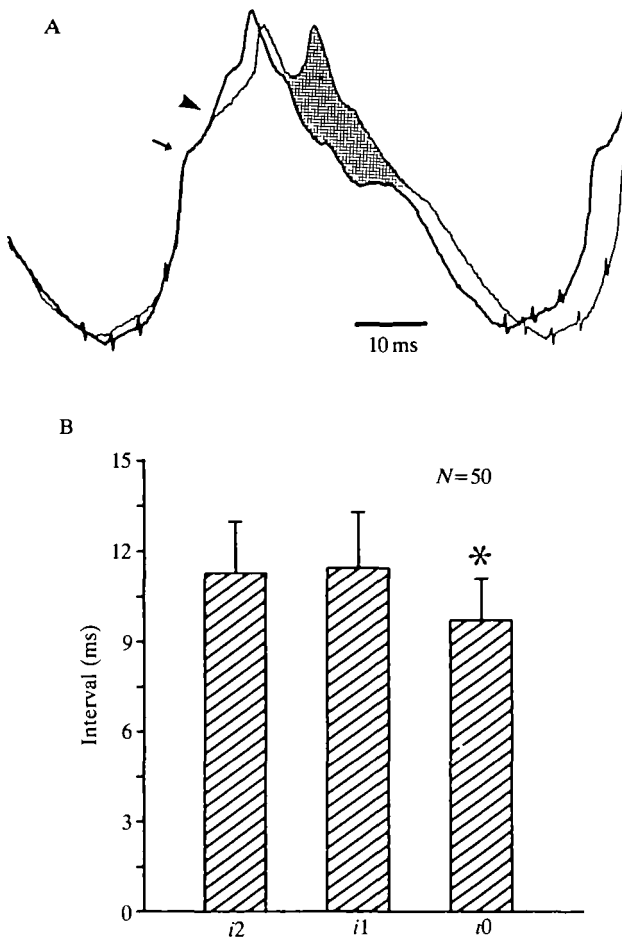
Fig. 9. Excitatory effect of the FSRs on the initial depolarization in elevator motoneurones. Experimental arrangement as for Fig. 8. (A) Superposition of the cycle immediately preceding the presentation of the stimulus train (thin trace) and the cycle immediately following the first stimulus train (thick trace). Note the reduction in the duration of both the peak (stippled) and base depolarizations, as described in Fig. 8. Note also that the interval between the first two spikes in each oscillation was reduced. The traces were aligned so that the first spike in each cycle (arrow) were superimposed. In this example the reduction in the interspike interval was associated with an additional excitatory input (onset indicated by arrowhead). (B) Bar graph showing that stimulation of the FSR resulted in a significant reduction in the interspike interval of the elevator burst. For 50 flight sequences the mean interspike interval was determined for the two cycles preceding the first stimulus train (i2 and i1) and the cycle immediately following the first stimulus train (i0). The FSR stimulation reduced the interval by about 1.5 ms (asterisk, $P<0.001$ paired Student's t-test), which was about 15 % of the interspike interval. Bars show standard deviations.

activity in M97 rarely reached 8 ms (Fig. 4). An important issue is whether this is true under all conditions. B. Möhl (personal communication) has observed that the elevation of the wings in tethered flying animals is lower than in free-flying animals (see Baker and Cooter, 1979, for measurements of latter). It would be expected that the activity in the FSRs would be more vigorous during free flight, and that the onset of FSR activity relative to depressor activity would be earlier than we have recorded in this investigation. Thus, under free flight conditions, it is conceivable that input from the FSRs could contribute to the activation of depressor motoneurones.

Until now the most detailed study on the timing of input from the FSRs has been made by Möhl (1985b). By recording directly from the sensory nerve 1D2 arising from the FSR/chordotonal organ complex he was able to characterize clearly the discharge pattern of the FSR in tethered flying animals. In general our data are consistent with the data presented by Möhl. However, Möhl did not describe the relationship between the timing of FSR activity relative to depressor activity as a function of wingbeat frequency, although he did present data showing that the onset of FSR activity preceded the onset of forewing depressor activity by about 10 ms (his Fig. 7). Examination of the data presented in this particular figure indicates that it may not represent the usual situation. First, there is doubt whether data in this figure were derived from an animal flying normally. This is because the delay between the onset of hindwing (M127) and forewing (M97) activity is about 20 ms, which is much greater than reported in other studies (Altman, 1975; Pearson and Wolf, 1987; Stevenson and Kutsch, 1987). Second, the raw data records in Möhl's paper (his Fig. 2) show that the timing of input from the FSRs varies as a function of the wingbeat frequency. As the wingbeat frequency declined, the onset of the FSR activity advanced relative to the onset of depressor activity. This is consistent with findings presented in this paper (Figs 2, 4). Finally, if allowance is made for the delay between the onset of activity in M127 and M97 (Möhl used activity in M127 as reference), it is clear that at high wingbeat frequencies the onset of FSR activity never preceded the onset of forewing depressor activity by 10 ms. Again, this is consistent with our observations.

Effect of FSR input of flight motoneurones

It has been known for some time that the FSRs make monosynaptic excitatory connections to wing depressor motoneurones (Burrows, 1975). However, these connections are weak and their role in the patterning of activity in depressor motoneurones has never been clear. There is no evidence that they contribute to the activation of depressor motoneurones in a flying animal, and none of our data indicates that the monosynaptic connections to depressor motoneurones are important in patterning flight motor activity. However, we did find that the FSRs can excite the depressor motoneurones *via* a stronger, polysynaptic pathway (Fig. 7). This polysynaptic excitation is distributed to depressor motoneurones on both sides of the meso- and metathoracic ganglia. The finding of these relatively powerful polysynaptic connections suggests that, from a functional viewpoint, the

polysynaptic pathways may be the most significant in mediating FSR effects onto depressor motoneurones. If this is true, it follows that the main action of the FSRs must be delayed relative to the burst of activity in the FSRs. This is because the polysynaptic EPSPs elicited by FSR stimulation had a minimum latency of 15 ms following the onset of a burst of activity in the FSRs and the peak of the EPSP occurred approximately 25 ms after the beginning of the burst (Fig. 7). Because the FSR bursts begin close to the peaks of the depolarizations in depressor motoneurones, the delayed effect of the polysynaptic pathways (if they are functional in flying animals) must occur close to the peak hyperpolarization of depressors. Consistent with this conclusion was our finding that electrical stimulation of the FSRs with a pattern similar to that occurring in flying animals caused a slowing of the repolarization, a reduction in the peak hyperpolarization and a faster initial depolarization in the next cycle of activity in depressor motoneurones (Fig. 6). From these observations, we conclude that the main action of input from the FSRs during flight is to oppose the hyperpolarization phase in depressor motoneurones. If this were true, it would mean that the degree of hyperpolarization is established by a balance between inhibitory input from the system generating the antagonistic elevator burst (Robertson and Pearson, 1985) and excitatory input from the FSRs. This type of antagonism has not been described in any other rhythmic motor system.

Another major finding of the present investigation was that input from the FSRs contributes an excitatory component to the initial depolarization in elevator motoneurones in flying animals (Fig. 9). This excitatory effect was manifested by a significant reduction in the interval between the first two spikes in the elevator burst, and often occurred in the absence of any noticeable effect on the amplitude of the depolarization. This excitatory contribution was not noticed in a previous investigation (Wolf and Pearson, 1988) because in that study the effect of FSR input on the initial depolarization in elevator motoneurones was assessed by measuring the area of the depolarization. The occurrence of an excitatory input from the FSRs to elevator motoneurones during flight is consistent with an earlier observation in *L. migratoria* where stimulation of the FSRs in quiescent animals results in a delayed, polysynaptic EPSP (see Fig. 2 in Reye and Pearson, 1987). [In *S. gregaria* these delayed EPSPs have not been observed (Burrows, 1975).] The timing of the onset and peak of these polysynaptic EPSPs is appropriate for them to contribute to the peak depolarization in the elevator motoneurones in a flying animal.

The finding of simultaneous excitation of both elevator and depressor motoneurones is consistent with the observation that the FSRs terminate on interneurones active in the elevator phase as well as interneurones active during the depressor phase (Reye and Pearson, 1988). Therefore, the present study resolves the question of why elevator interneurones should receive excitatory input from the FSRs. Presumably these interneurones form part of the system responsible for exciting elevator motoneurones in a flying animal. In addition, some of them may be involved in opposing the hyperpolarization phase in depressors.
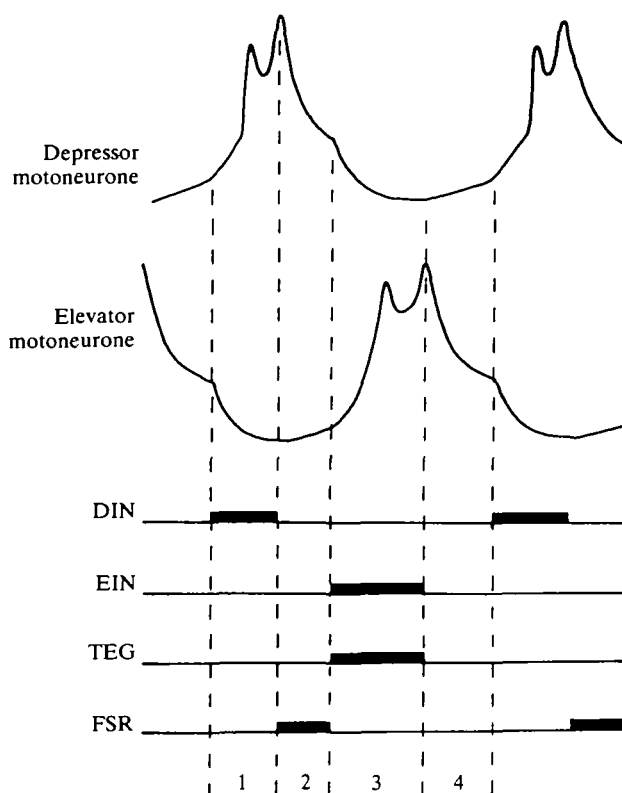
Fig. 10. Diagram illustrating the four phases of the flight cycle. The top two drawings illustrate the main features of the oscillations in the membrane potential of depressor and elevator motoneurones. The bottom four traces indicate the time of activity in depressor interneurones (DIN), elevator interneurones (EIN), the hindwing tegulae (TEG) and the forewing stretch receptors (FSR). Further explanation in text.

Model for sensory regulation of flight motor activity

The results of this and previous investigations allow us to propose a mechanistic explanation for the functioning of proprioceptive input in the patterning of motor activity for flight. Before describing this model it is first necessary to note that the flight cycle can be divided into four distinct phases (Fig. 10) on the basis of data from previous studies on the profiles of synaptic input to flight motoneurones (Wolf and Pearson, 1987) and interneurones (Wolf and Pearson, 1989). The first phase is defined by the activation of depressor motoneurones. The onset of the rapid depolarization in depressors in this phase corresponds to the onset of the late repolarization in elevator motoneurones. During the second phase the depressors repolarize and the elevators slowly depolarize. At this time there is no spike activity in either elevators or depressors. The third phase corresponds to the time of activation of the elevators. A hyperpolarizing inflection usually occurs in depressors at the time of onset of the rapid depolarization in elevators. During the

fourth phase the elevators repolarize to a distinct plateau and the depressors slowly depolarize. The division of the flight cycle into four phases based on motoneuronal recordings is supported by recordings from interneurones. In intact tethered flying animals most of the phasically active interneurones discharge at times corresponding to phase 1 or phase 3 (see Wolf and Pearson, 1989, for data). No interneurones have been found to be phasically active in phases 2 and 4, although this does not mean that interneurones with this pattern of activity do not exist. Also shown diagrammatically in Fig. 10 is the timing of the input from the hindwing tegulae (TEG) and forewing stretch receptors (FSR). The tegulae input occurs during phase 3 and is responsible for initiating activity in elevator interneurones and motoneurones during this phase (Wolf and Pearson, 1988). The FSR input occurs during phase 2, as described in this study.

In the scheme we propose for explaining the sensory regulation of the motor pattern (Fig. 11) we have made three major assumptions. (1) Interneurones are organized into two groups: elevator interneurones (EIN) and depressor inter- neurones (DIN). These two groups are connected *via* mutual inhibitory pathways, and each group of interneurones contains members that provide excitatory input to flight motoneurones (Robertson and Pearson, 1985). (2) Bursts in the elevator motoneurones are produced by phasic sensory input from the hindwing tegulae (Pearson and Wolf, 1988; Wolf and Pearson, 1988) with the FSRs contributing an excitatory component. [Another possibility is that elevator bursts are generated centrally and simply initiated by tegula input. In this case central mechanisms would be responsible for terminating elevator activity (see Wolf and Pearson, 1988).] (3) Bursts in the depressor interneurones are generated centrally, i.e. initiated following a period of passive depolarization (occurring during phase 4) and terminated as a result of intrinsic central mechanisms (Wolf and Pearson, 1987*a*, 1988, 1989).

With these assumptions we can now proceed to describe the sequence of events involved in the generation of the flight motor pattern (Fig. 11). In Fig. 11 the active element(s) in each phase is(are) shown with thick lines.

Phase 1. A burst of activity is centrally generated in depressor interneurones. This causes a rapid depolarization and activation of depressor motoneurones, and at the same time an inhibition of elevator interneurones and a complete repolarization of elevator motoneurones.

Phase 2. During this phase the FSRs are active, the depressors are repolarizing and the elevators are slowly depolarizing, owing to the removal of excitatory and inhibitory input, respectively. No interneurones have been found to be phasically active in this phase in intact animals (Wolf and Pearson, 1989).

Phase 3. The depression of the wings that occurs during phases 1 and 2 causes excitation of the hindwing tegulae. This phasic signal from the tegulae excites the elevator interneurones and, as a consequence, inhibits depressor interneurones. During this phase input from the FSRs has two effects *via* a delayed (*D*) excitatory pathway (we presume that the delay is due to the time required to activate interneurones). The first is to contribute to the excitation of elevator neurones,
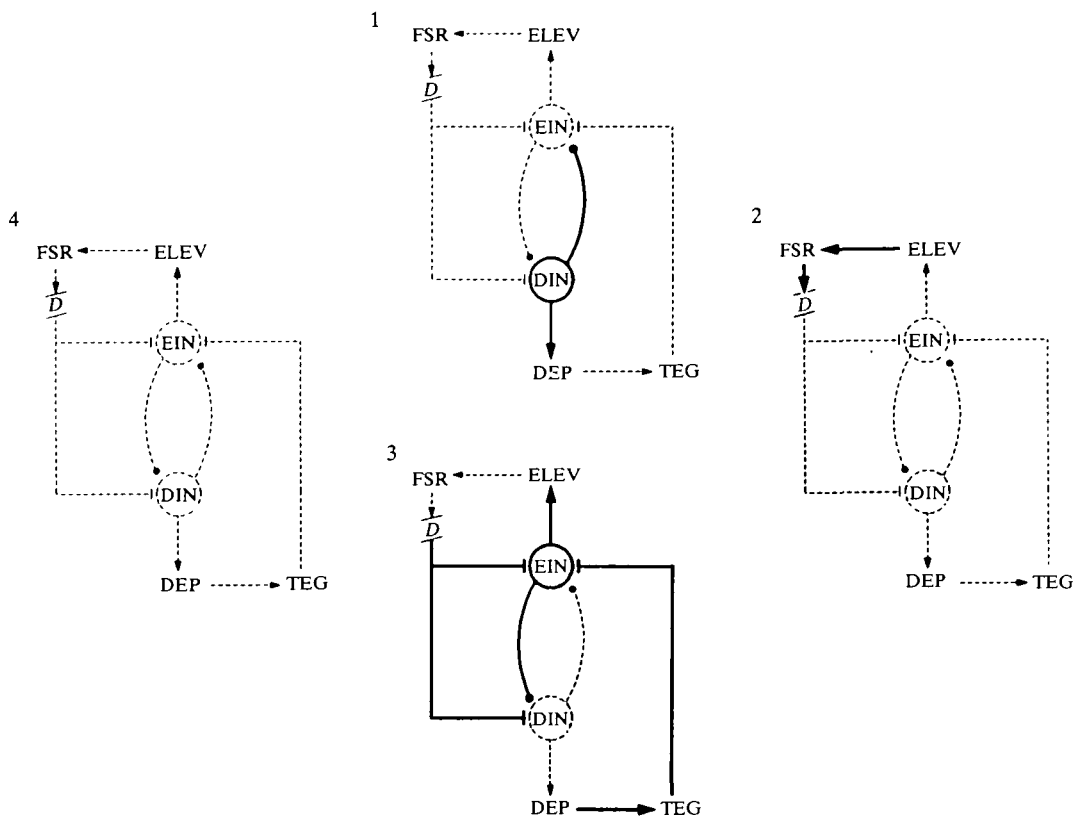
Fig. 11. Diagram illustrating the mechanism by which input from the FSRs and hindwing tegulae (TEG) contribute to the patterning of activity in flight moto-neurones. Each of the four diagrams corresponds to one of the four phases illustrated in Fig. 10. The thick lines in each diagram indicate the element(s) of the circuit that is(are) active in that phase. DEP, depressor motoneurones; ELEV, elevator moto-neurones; D, delayed excitatory pathway. Further explanation in text.

and the second is to excite depressor interneurones and so oppose the hyperpolar-ization in these neurones. The effect of the latter is to reduce the peak hyperpolarization in depressor motoneurones (Fig. 6). The termination of activity in elevator neurones during this phase is assumed to be the result of the termination of activity in tegula afferents.

Phase 4. The elevators repolarize to a plateau and the depressors slowly depolarize owing to removal of excitatory and inhibitory inputs, respectively. No afferent input or interneuronal activity is associated with this phase (this study; Wolf and Pearson, 1989). Phase 4 is terminated when the depressor burst generator recovers from the inhibitory input it received during phase 3. In the event that no input from the FSRs occurs during phase 3 the depressor system begins its depolarization from a more negative potential and takes longer to reach threshold for burst generation at the end of phase 4.

The model in Fig. 11 provides a qualitative understanding of how afferent input is involved in the patterning of motor activity for flight. In particular, it suggests a mechanism for how the FSRs could function to regulate wingbeat frequency. Any tendency to delay the onset of depressor activity (due, for example, to less central input to the pattern-generating network) would result in a larger elevation of the wings and more activity in the FSRs (see Möhl, 1985*b*, for the relationship between wing elevation and FSR activity). The increase in FSR activity would oppose the hyperpolarization of depressors and lead to an earlier onset of the next depressor burst, thus compensating for the delay in the onset of the previous depressor depolarization. The model also explains why removal of the FSRs decreases the wingbeat frequency. The absence of input from the FSRs leads to an increase in the magnitude of the hyperpolarization phase in depressor neurones and thus delays the generation of the next depressor burst. This explanation differs from an earlier proposal that the FSRs elevate wingbeat frequency by suppressing a late depolarization in elevator motoneurones (Wolf and Pearson, 1988). Although the suppression of the late depolarization may have some role in regulating wingbeat frequency, this must be regarded as minor because the suppression is restricted to the peak depolarization and the reduction in this peak depolarization is poorly correlated with the reduction in cycle period (Fig. 8C).

An obvious avenue for future investigations is to develop a more quantitative description of the interactions of the elements illustrated in Fig. 11. This may then allow the development of a computer simulation of the network and more rigorous testing of hypotheses regarding the function of wing proprioceptors.

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