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A Preparation for the Intracellular Analysis of Neuronal Activity During Flight in the Locust

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Summary. 1. A simple preparation in *Locusta migratoria* is described which allowed intracellular recording from interneurons and motoneurons in the thoracic ganglia during flight. Flight activity could be evoked in approximately 95% of the preparations by wind stimulation of the head.

2. Intracellular recordings from the neuropile processes of motoneurons showed that spike activity was produced by large amplitude (up to 25 mV) oscillations in membrane potential. Inhibitory input contributed to the repolarizing phase in both elevators and depressors.

3. Elevator and depressor motoneurons could be distinguished by their initial response to wind stimulation of the head. Elevators were depolarized beyond their threshold whereas most depressors were hyperpolarized.

4. Numerous interneurons phasically modulated during flight were identified in both the meso- and metathoracic ganglia. Some of these interneurons were found to make short-latency excitatory or inhibitory connections to flight motoneurons and contribute significantly to the rhythmic synaptic input to motoneurons.

5. A pair of interneurons was identified which, when depolarized by current injection, decreased the frequency of the flight rhythm by up to 50%. These interneurons were phasically modulated only during the expression of the flight rhythm as indicated by the flight activity of motoneurons.

6. We conclude that the flight rhythm is generated primarily at the interneuronal level, that spiking premotor interneurons are largely responsible for driving the motoneurons, and that the flight oscillator is not continuously active.

Abbreviations: *DL* dorsal longitudinal (muscles); *EMG* electromyogram

Introduction

One of the major problems in neurobiology today is to characterise the processes which determine the time of activation of any particular motoneuron relative to any other. The sequential activation of different motoneurons underlies the orderly contraction of different muscles, and this in turn underlies behavior. The central origin of motor output patterns is now well established (Grillner 1977; Selverston 1980), particularly for rhythmical behaviors (Delcomyn 1980). Thus for many motor systems we are now faced with the problem of determining the cellular basis for central patterning and the modulatory action of sensory input on the output pattern.

The locust flight system was one of the first in which it was demonstrated convincingly that sensory feedback was unnecessary for organizing the basic rhythm (Wilson 1961). However, over the past 20 years we have gained little knowledge of the mechanisms for centrally generating the flight rhythm. Although there is some data suggesting that delayed coupling between flight motoneurons may be important in the patterning of flight activity in the locust (Burrows 1973a), and also in the dragonfly (Simmons 1977), it is now quite obvious that the basic rhythm is generated at an interneuronal level (Burrows 1977). At present, however, no flight interneurons have been identified in the thoracic ganglia of any insect. This is largely due to the difficulty of intracellularly penetrating interneurons in flying animals, as well as the low probability of evoking flight activity in the preparations used to date.

In this paper we describe a simple preparation in *Locusta migratoria* in which the flight rhythm can be easily evoked after extensive dissection, and in which intracellular recordings can readily be made from interneurons and motoneurons. Using this preparation we have confirmed that the flight rhythm can

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be generated in the absence of sensory input from wing proprioceptors, and identified numerous ($n=25$) interneurons whose activity is modulated strongly in phase with flight motor activity. By simultaneously recording from an interneuron and a motoneuron we have shown that some of these modulated interneurons make short-latency connections with flight motoneurons. Since these interneurons provide a significant synaptic input to motoneurons our data support previous conclusions that flight motor activity is generated primarily at an interneuronal level. This conclusion is strengthened by our finding of an interneuron which, when depolarized by current injection, strongly influenced the flight frequency. None of our data indicate that the flight oscillator is active in the absence of an overt expression of flight activity in the motoneurons.

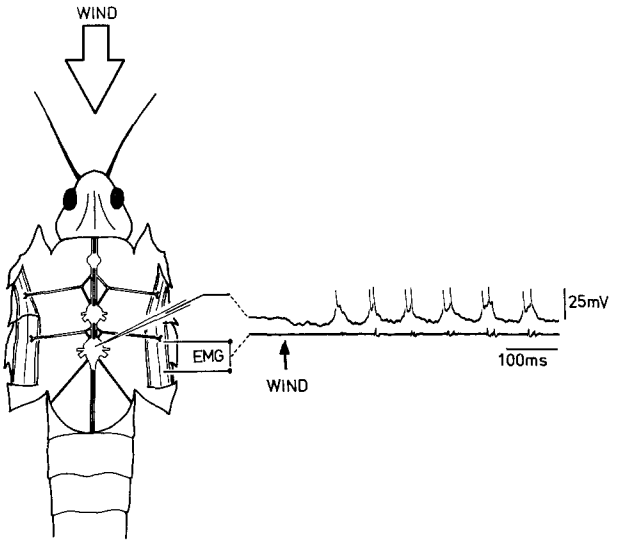

Fig. 1. Schematic diagram of the preparation for intracellularly recording from neurons during flight. The three thoracic ganglia were exposed in a locust whose wings and legs had been amputated. Nerves 3, 4 and 5 of the meso- and metathoracic ganglia were cut to reduce inhibitory input from the periphery and to ensure maximum stability of the ganglia. The nerves to the dorsal longitudinal muscles were left intact to maintain an EMG monitor of depression during flight. Intracellular recordings were taken from the neuropile segments of neurons in the meso- and metathoracic ganglia. In the example shown in this figure the recording was made from a dorsal longitudinal motoneuron in the metathoracic ganglion. Rhythmical neuronal activity was induced by blowing wind on the head of the locust. In this and all subsequent traces an arrow underneath the trace indicates the time of onset of wind stimulation, and the dorsal longitudinal EMG monitors the time of occurrence of wing depressor activity. Impaled neurons were filled with Lucifer yellow for subsequent identification

Materials and Methods

Mature adult *Locusta migratoria* were obtained from a long established colony at the University of Alberta. All experiments were performed at room temperature (22–24° C).

Dissection. The wings and the legs were amputated and the animal pinned dorsal side up to a cork board. The thorax was then opened with a midline incision and pinned in such a way as to allow access to the body cavity. Care was taken at this point not to spread the thoracic cuticle too broadly, as this tended to abolish flight. The thoracic ganglia were exposed by removing the overlying viscera, muscle and connective tissue, and the meso- and metathoracic ganglia were supported on a flat stainless steel plate. To reduce inhibitory input from the periphery and to ensure maximum stability of the ganglia, nerves 3, 4, and 5 (numbering after Campbell 1961) on the right and left sides of both the meso- and metathoracic ganglia were cut about 0.5 mm from the ganglia. Nerves 1 of both ganglia and nerves 6 of the mesothoracic ganglion were usually left intact in order to maintain an output connection with some flight muscles (the dorsal longitudinals, DL, which are indirect depressors). A pair of copper wires, insulated except at the tip, was inserted into one of these muscles, usually the right metathoracic DL, to provide an electromyographic (EMG) monitor of the time of depressor motor activity. In some preparations the abdominal connectives posterior to the metathoracic ganglion and all the lateral nerves of the pro-, meso- and metathoracic ganglia were cut. This resulted in a preparation in which all input from thoracic and abdominal afferents was removed. A similar preparation was used by Camhi and Hinkle (1972) to demonstrate that a high frequency bursting pattern in abdominal motoneurons was derived centrally. The preparation was continuously perfused with saline (NaCl, 147 mM; KCl, 10 mM, $CaCl_2$, 4 mM; NaOH, 3 mM; HEPES buffer, 10 mM).

With this preparation flight activity was evoked by blowing air on the animal's head from a wide (internal diameter = 7 mm) tube placed approximately 2 cm directly in front of the head. The duration of the flight sequences was usually kept to less than 5 s by brief application of the wind stimulus. Much longer sequences of flight (up to 1 min) could be evoked by continuously blowing on the head and decreasing the ambient light intensity. A schematic diagram of the experiment set-up is shown in Fig. 1.

Recording and Staining. Intracellular recordings were taken from the neuropile processes of flight neurons in both ganglia using glass microelectrodes pulled such that when filled with 1 M potassium acetate they had resistances of approximately 50 megohms. The tips of the electrodes were filled with 4% Lucifer yellow (Stewart 1978) and the shaft back filled with 0.5 M lithium chloride. These electrodes had resistances over 100 megohms. Dye was injected with constant hyperpolarizing currents (between 5 and 10 nA) for about 10 min and left to migrate for a further 30 min. The ganglia were then removed, fixed in 4% paraformaldehyde (pH = 7.2) for 30 min, dehydrated in ethanol and cleared in methyl salicylate. Filled neurons were viewed on a Leitz fluorescence microscope and immediately photographed and drawn.

Intracellular and extracellular activity was amplified and displayed conventionally, and stored on magnetic tape for subsequent filming and analysis.

Identification of Flight Motoneurons. Since the axons of flight motoneurons were cut, motoneurons were identified by their anatomical features after staining with Lucifer yellow. The main morphological features were determined in a series of preliminary experiments in which penetration of a particular motoneuron could be confirmed on purely physiological grounds. In these experiments, the sides of the thorax were flattened, nerves 3, 4 and 5 of both ganglia were left intact, and the medial row of flight muscles detached at their dorsal insertions so that both medial and lateral flight muscles could be observed. In this situation, the rhythmical activity described above (Fig. 1) did not occur. However, a penetrated

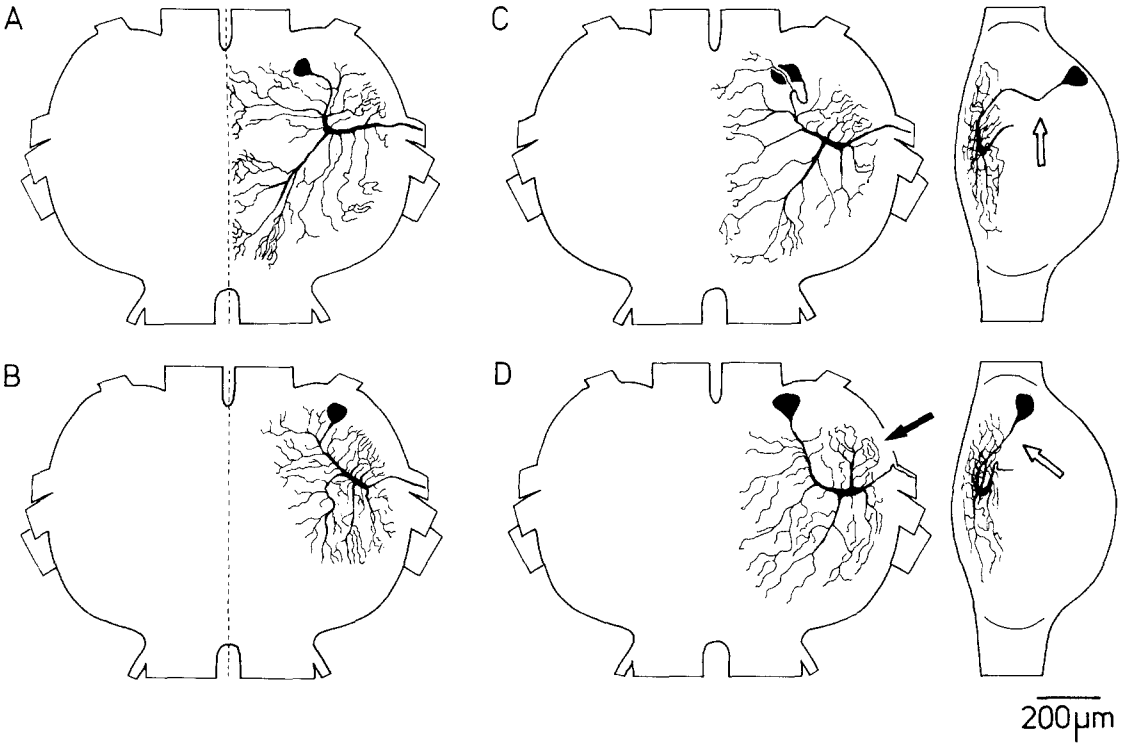

Fig. 2A–D. Morphology of flight motoneurons. Drawings from the dorsal aspect (**A** and **B**) and dorsal and lateral aspects (**C** and **D**) of flight motoneurons in the mesothoracic ganglion filled with Lucifer yellow (anterior is towards the top). **A** Tergosternal motoneuron. **B** Anterior tergocoxal motoneuron. Note the more restricted branching pattern compared to the tergosternal. **C** First basalar motoneuron. Note the deep (ventral) cell body and the primary neurite (*open arrow*) arising sharply from the main neuropile segment. **D** Second basalar motoneuron. Note the shallow location of the cell body and the path of the primary neurite (*open arrow*). Second basalar motoneurons have a prominent anteriorly directed branch arising from the main neuropile process (*filled arrow* in **D**) which is not seen in first basalar motoneurons

flight motoneuron could be identified by the 1:1 correspondence of motoneuron spikes evoked by current injection with a muscle's twitch contractions. In these preparations the flight muscles were well separated and there was no difficulty in positively identifying the muscle which was contracting. The anatomy of the flight motoneurons is similar to that previously described for *Schistocerca gregaria* (Bentley 1970) and *Chortoicetes terminifera* (Burrows 1973b; Tyrer and Altman 1974) (see for example Fig. 2).

During experiments with the preparation exhibiting rhythmical output, identification of a motoneuronal penetration progressed in several stages. First, motoneuronal and interneuronal penetrations could be reliably differentiated on the grounds that motoneurons have a larger resting membrane potential, a larger spike amplitude and duration, and a lower intraburst spike frequency (compare Figs. 3 and 7). Second, a motoneuron was classified as either an elevator type or a depressor type, depending on the time of its burst relative to the rhythmical activity in the dorsal longitudinal (depressor) EMG. Occasionally the process of identification was halted at this stage and the motoneurons classified simply as either elevators or depressors. Finally, the motoneuron was filled with Lucifer yellow and its morphology compared with that of flight motoneurons identified in the preliminary study and in other species of locust.

Results

To establish that the rhythmical output produced by the preparation described above is indeed a manifestation of the centrally generated flight rhythm, the essential characteristics of the flight pattern must be apparent. The behavior must be elicited by the appropriate stimuli, oscillate at the expected frequency, and show the correct patterns of alternation between antagonists and phase lag between segmental homologues (hind leading fore). All these criteria were fulfilled. The stimulus for the normal flight behavior is wind on the head in the absence of tarsal contact (Weis-Fogh 1956b). In our preparation tarsal contact was eliminated by leg amputation and wind stimulation elicited a rhythmic motor output in almost all preparations (approximately 95%). The mean frequency of activity observed in different preparations was 10.4 Hz (SD = 1.6, $n = 50$) which is about half the frequency in free flying locusts (Baker et al. 1981). This decrease in frequency is presumably due to a functional deafferentation since a similar reduction was found by Wilson (1961) following deafferentation in *Schistocerca gregaria*. The characteristic pattern of motor activity produced by our preparation consisted of accelerating bursts of activity in elevator motoneurons followed by similar bursts in depressor motoneurons followed by a variable silent period,

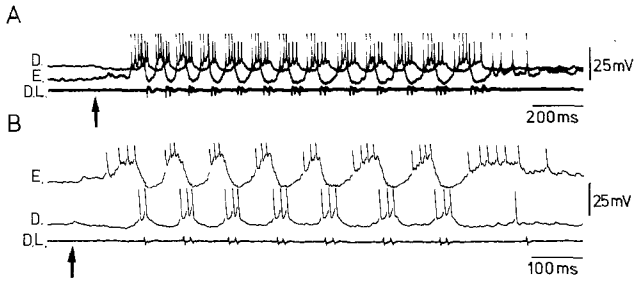

Fig. 3A, B. Simultaneous intracellular recordings from unidentified depressor (*D*) and elevator (*E*) motoneurons in two different preparations. Note the delay between the end of the depressor bursts and the beginning of the elevator bursts. The bottom trace in **A** and **B** (*DL*) is the EMG from the dorsal longitudinal muscles

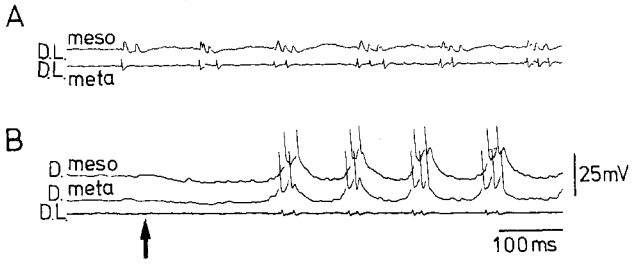

Fig. 4A, B. Phase lag between metathoracic (hindwing) and mesothoracic (forewing) activity during a flight sequence. **A** Simultaneous EMG recordings from the ipsilateral dorsal longitudinal muscles in the mesothoracic (*DL meso*) and metathoracic (*DL meta*) segments shows that metathoracic activity leads by about 5 ms. **B** Similar phase lags can be observed in simultaneous intracellular recordings from unidentified ipsilateral depressor motoneurons of the two ganglia (D meso and D meta)

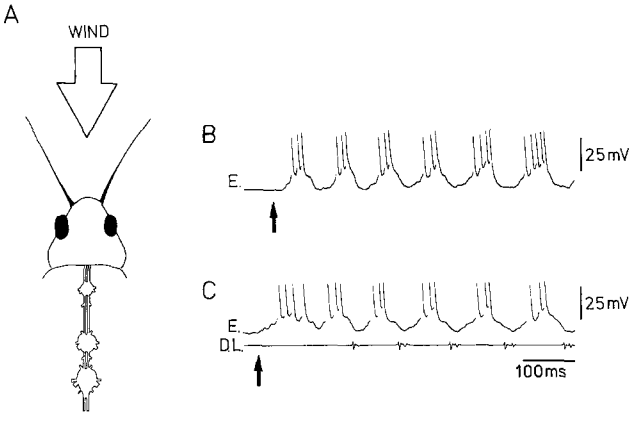

Fig. 5A–C. Flight activity following removal of all afferent input from thoracic and abdominal segments. **A** Deafferented preparation in which the abdominal connectives were cut posterior to the metathoracic ganglion and all the nerve roots of the thoracic ganglionic chain were cut (compare with Fig. 1). **B** Intracellular recording taken from an identified mesothoracic tergosternal motoneuron (*E*) in a deafferented preparation showing rhythmic activity induced by wind stimulation of the head (onset indicated by *arrow*). **C** Flight activity of an identified mesothoracic tergosternal motoneuron (*E*) recorded from a preparation with a less extensive deafferentation (Fig. 1). Note the similarity of the recordings taken from the two preparations

and a sequence of flight activity always started with a relatively long elevator burst (Fig. 3). All these features are characteristics of the motor activity in intact animals (Wilson 1961; Pond 1972). Finally EMG recording from the dorsal longitudinal (DL) muscles of the two segments in preparations set up exactly as for the intracellular recording showed a lag of meso-DL activity following meta-DL activity by approximately 5 ms (Fig. 4A), this being similar to that seen in intact preparations (5 to 10 ms, Weis-Fogh 1956a; Baker and Cooter 1979). In particularly good double intracellular penetrations of individual motoneurons a similar lag was also apparent (Fig. 4B). From all these observations we conclude that the motor patterns generated in our preparation are true manifestations of the flight motor activity, although the overall frequency is lower than in normal intact animals. Furthermore these patterns reflect the central component of the flight motor system since similar patterns were generated following complete deafferentation of the thoracic nerve cord (Fig. 5).

Input to Flight Motoneurons During Flight

Examination of the recordings obtained intracellularly from different motoneurons during flight revealed that in both elevator and depressor motoneurons the bursts of spikes were produced by fairly smooth oscillations of the membrane potential. The amplitude of these oscillations in membrane potential were commonly 15 mV and could be as large as 25 mV. It was apparent that several excitatory and inhibitory synaptic inputs cooperated to shape the waveform (Fig. 6). In both elevators and depressors a depolarizing (excitatory) synaptic input produced the burst, and repolarization following a burst was due, in part, to inhibitory synaptic input (see also Figs. 1, 3–5, 8 and 10). In some instances while recording simultaneously from an elevator and depressor motoneuron it was possible to observe single cycles of flight. In all these cases the phasic depolarization of elevators was accompanied by a phasic hyperpolarization of depressors, and vice versa.

Two other features of the pattern of synaptic input to motoneurons are noteworthy. Firstly, there was an asymmetry in the temporal pattern of synaptic input to elevator and depressor motoneurons. Elevator activity immediately preceded depressor activity which was followed by a delay before the next cycle of elevator activity (Fig. 3). Secondly, the elevator and depressor motoneurons responded differently to the onset of the wind stimulus to the head. All elevators were strongly depolarized beyond threshold whereas most depressors were hyperpolarized. Occasionally a depressor motoneuron was weakly depolar-

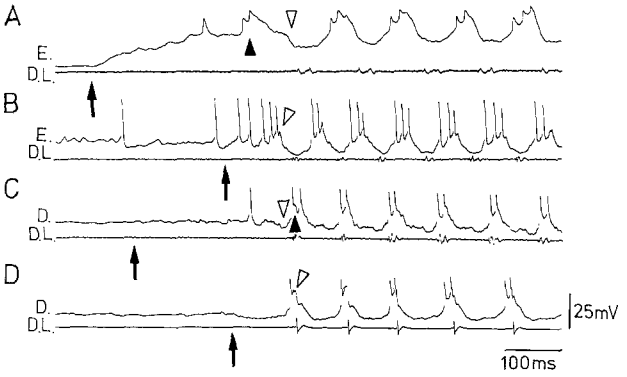

Fig. 6A–D. Phasic inputs underlying the membrane potential waveform of motoneurons during flight. *Arrows:* time of onset of wind stimulation of the head. *Filled and open arrowheads:* time of occurrence of phasic depolarizing and hyperpolarizing inputs respectively for the first cycle of each sequence. These inputs become more difficult to discern once the flight rhythm is established. **A** Mesothoracic tergosternal motoneuron showing depolarizing input to induce a burst and a hyperpolarizing input at the time of antagonist firing. These are superimposed on the pronounced plateau of depolarization caused by wind stimulation. **B** Mesothoracic tergosternal motoneuron in another preparation showing hyperpolarizing input at the termination of a burst. Note that the recorded amplitudes of both spikes and the oscillations are variable in different preparations (compare **A** and **B**). **C** Mesothoracic second basalar motoneuron showing depolarizing input to induce a burst and hyperpolarizing input at the time of antagonist firing. **D** Metathoracic subalar motoneuron showing hyperpolarizing input to terminate a burst

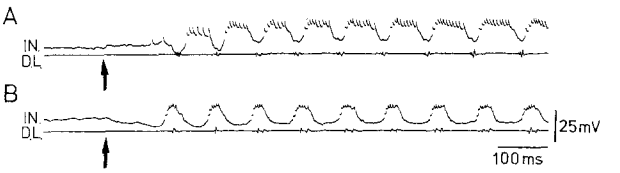

Fig. 7A, B. Examples of interneurons phasically active during flight. **A** An interneuron in the metathoracic ganglion which fired in high frequency bursts in phase with elevator motoneurons. **B** An interneuron in the metathoracic ganglion which fired in high frequency bursts in phase with depressor motoneurons. Note that the elevator-type interneuron (**A**) was depolarized by wind on the head (onset indicated by *arrow*) and during flight, whereas the depressor-type interneuron (**B**) was hyperpolarized by wind and during flight

ized (Fig. 6C) but never beyond threshold. The synaptic input to the flight motoneurons from wind stimulation appeared to persist throughout flight sequences such that the oscillations in membrane potential in elevators and depressors were superimposed on a maintained depolarization and hyperpolarization respectively (Figs. 3 and 6). However, this pattern night result from varying levels of phasic depolarizing and hyperpolarizing input.

Although the general pattern of synaptic input to flight motoneurons could be clearly distinguished, it was impossible to follow individual postsynaptic potentials from a single presynaptic neuron throughout the flight sequence. Both the depolarizing and hyperpolarizing synaptic inputs appear to be made up of many small asynchronously occurring PSPs. Furthermore it was impossible to determine whether the same interneurons were acting on different motoneurons during flight, although common PSPs were often seen in different motoneurons when the animal was not flying (see also Burrows 1975a, b). From these observations we conclude that very little useful information about the mechanisms for the generation of the flight rhythm can be obtained by intracellularly recording only from motoneurons. Nor can information be obtained from injection of current into motoneurons since application of currents of up to 20 nA during flight did not perturb the flight rhythm in any obvious way. That such current injection was an effective stimulus was observed in non-flying animals where similar current injections of varying strengths into flight motoneurons could generate activity of frequencies up to and exceeding that observed during flight.

Rather than seeking further details of synaptic events in motoneurons the most obvious strategy for gaining additional insight into the generation of the flight pattern was to identify the neurons producing the PSPs in motoneurons during flight, and search for interneurons which, when perturbed by current injection, influence the flight rhythm. Our results using this strategy are presented in the following section.

Interneuronal Activity During Flight

Many interneurons whose spike activity (or membrane potential) was rhythmically modulated during flight were penetrated within the neuropile of the meso- and metathoracic ganglia. Two examples of rhythmic interneuronal activity are shown in Fig. 7. The characteristic feature of interneuronal activity was the prolonged high frequency burst during the depolarizing phase. Typically spiking interneurons produced 5 to 10 spikes/burst at very high intraburst frequencies usually between 200 and 250 impulses/s. This is to be compared to motoneurons which usually produced 2 to 3 spikes/burst at frequencies of about 60 to 80 impulses/s. All the rhythmically active spiking interneurons we identified ($n=25$) had this characteristic pattern of activity. These interneurons discharged either in-phase with elevator activity or in-phase with depressor activity and could therefore be segregated into two distinct groups. We did not attempt a classification of interneurons within each of these groups since there were no immediately obvious differences. In the following sections we make no attempt to describe all the rhythmically active inter-

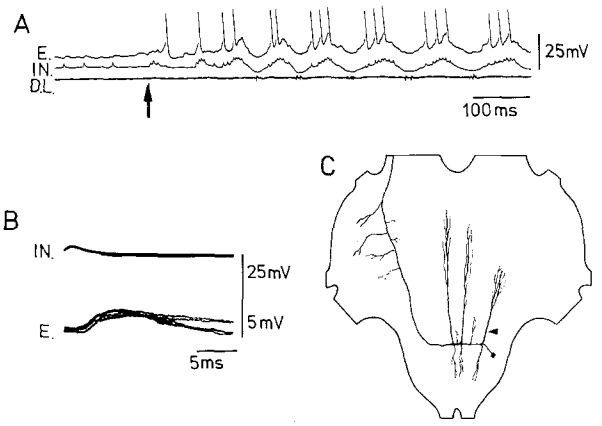

Fig. 8A–C. Activity and structure of an elevator type interneuron which had an excitatory projection to an elevator motoneuron. **A** Simultaneous intracellular recordings from an unidentified elevator motoneuron (*E*) in the mesothoracic ganglion and the interneuron (*IN*). In response to wind stimulation of the head (*arrow*) the interneuron was depolarized to fire and during flight it fired in high frequency bursts in phase with elevator motoneurons. **B** Successive oscilloscope sweeps triggered by the rising phase of the interneuronal spike showed that each spike in the interneuron was followed after a short constant latency (3 ms) by a small (1–2 mV) EPSP in the elevator motoneuron. This is also evident in **A**, particularly before flight was induced. **C** The interneuron had a small cell body in the posterior lateral region of the metathoracic ganglionic mass within the fused second abdominal ganglion and characteristic longitudinal arborizations. The axon travelled to the mesothoracic ganglion in the lateral edge of the connective contralateral to the cell body. The structure anterior to this is unknown. *Arrowhead:* approximate site from which the recordings of **A** and **B** were taken

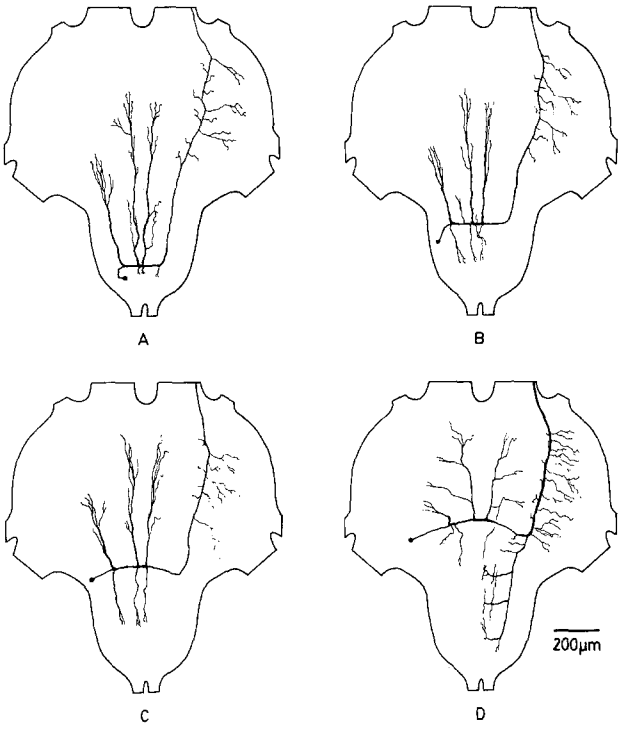

Fig. 9A–D. Structure of 4 elevator-type interneurons which appear to be serial homologues. All of these interneurons gave high frequency bursts in phase with elevators during flight and interneurons **B** and **D** were shown to form short latency excitatory connections to elevator motoneurons (see example in Fig.8). The interneurons depicted in **A**, **B** and **C** were located in the fused third, second and first abdominal ganglia. The interneuron depicted in **D** was located in the true metathoracic ganglion. Note the structural similarities including the longitudinal arrangement of the dendrites and the location of the axons in the extreme lateral edge of the connective contralateral to the cell bodies

neurons, but only give a description of those found to produce short latency PSPs in flight motoneurons or to alter the flight rhythm.

An example of an elevator interneuron making a short latency excitatory connection to elevator motoneurons is shown in Fig. 8. This neuron had a small (30 µm diameter) cell body located in the lateral portion of the fused second abdominal ganglion, a number of arborizations in the dorsal neuropile running parallel on either side of the midline and a small diameter axon which ascended the thoracic nerve cord in the lateral edge of the meso-metathoracic connective. Numerous small branches arose from the axon as it ascended through the metathoracic ganglion. These branches terminated in dorsal neuropile regions containing the processes of flight motoneurons. The structure in the meso- and prothoracic ganglia was not determined, and the final destination of the axon is unknown. Spikes in this interneuron produced EPSPs after a short and constant latency in at least one elevator motoneuron in the mesothoracic ganglion (Fig. 8 B). The short latency of the EPSPs suggests the connection was monosynaptic but the possibility of an intercalated interneuron can not be excluded. The amplitude of the EPSPs was about 1.5 mV which was small compared to the amplitude of the depolarizations (15 mV) in the elevator motoneuron during flight.

Other elevator interneurons with structures very similar to that shown in Fig. 8 were also found in the fused third and first abdominal ganglia (Fig. 9 A, C) and an elevator interneuron sharing some of the main features was found in the metathoracic ganglion (Fig. 9 D). The latter interneuron also produced EPSPs after a short and constant latency in a mesothoracic tergosternal motoneuron. These EPSPs were quite small in amplitude (1 to 2 mV).

Phasically active interneurons with inhibitory connections to flight motoneurons were also found. For example, the interneuron shown in Fig. 10 A produced short latency IPSPs in an unidentified elevator motoneuron in the metathoracic ganglion and fired in phase with depressors during flight. This interneuron had a small soma (35 µm diameter) in the posterior medial region of the mesothoracic ganglion.

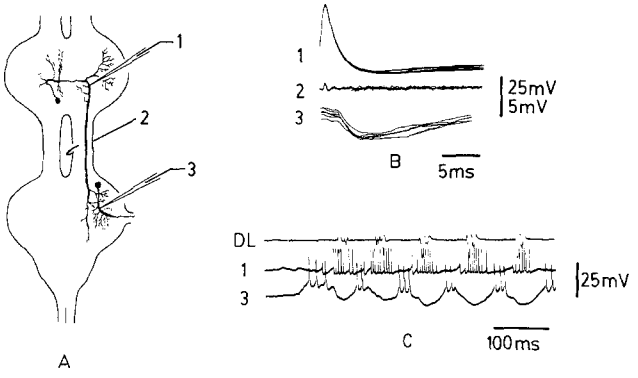

Fig. 10A–C. Structure and activity of a depressor-type interneuron which had an inhibitory projection to an elevator motoneuron. **A** Drawing of the interneuron in the meso- (*top*) and metathoracic (*bottom*) ganglia and the arrangement of recording electrodes. The interneuron had a small cell body in the posterior medial region of the mesothoracic ganglion. The neuropile segment crossed the midline and the axon had branches unilaterally in both the meso- and metathoracic ganglia. Simultaneous intracellular recordings were made from the interneuron (*1*) and an elevator motoneuron (*3*), and the activity in the descending axon of the interneuron monitored by recording extracellularly from the meso-metathoracic connective (*2*). **B** Inhibitory connection to an elevator motoneuron. Successive oscilloscope sweeps triggered by the rising phase of the interneuronal spike showed that each spike recorded intracellularly (*1*) was followed after a short constant latency (2–3 ms) by an IPSP (3 mV) in a metathoracic elevator motoneuron (*3*). **C** In a different preparation it was seen that the interneuron (*1*) fired in phase with depressors during flight and the highest frequency activity of the interneuron was coincident with a prominent hyperpolarization of an elevator motoneuron (*3*)

Its primary neurite was directed anteriorly and laterally before turning into the transversely orientated main neuropile process. Before crossing the midline this process gave off numerous anteriorly and posteriorly directed branches. The axon arose close to the midline and bifurcated to give one branch terminating in the dorsal neuropile region of flight motoneurons in the mesothoracic ganglion, and the other descending in the connective contralateral to the cell body to terminate in the dorsal neuropile region of flight motoneurons in the metathoracic ganglion. Again we cannot be certain that the inhibitory connection made by this interneuron was monosynaptic but the short and constant latency of the IPSPs and the location of its terminal processes are consistent with the existence of a direct connection.

Although we have now identified more than 20 interneurons whose activity is rhythmically modulated during flight we have so far found only one that has any obvious influence on the flight rhythm when injected with current. This interneuron is a depressor type (Fig. 11A) and has a small cell body (30 μm diameter) located postero-laterally in the metathoracic ganglionic mass within the fused first abdominal ganglion. The primary neurite crosses the midline and gives off extensive longitudinal arborizations in the dorsal neuropile. The axon arises from near the middle of the neuropile segment and ascends towards the mesothoracic ganglion in the medial por-

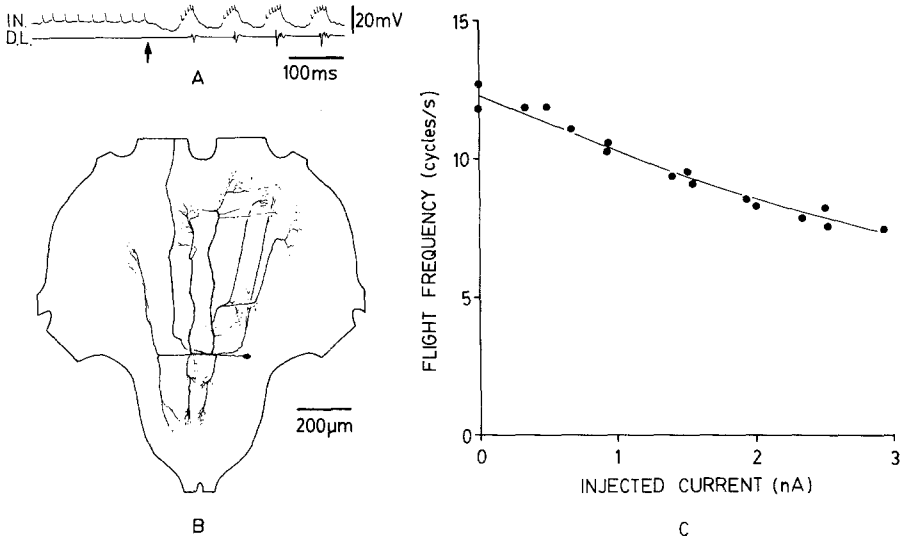

Fig. 11A–C. Activity and structure of a depressor-type interneuron depolarization of which altered the flight frequency. **A** The interneuron (*IN*) fired high frequency bursts in phase with depressor motoneurons (*DL*) during flight. Note that prior to full expression of flight there was no indication of any membrane potential oscillations at the flight frequency. **B** The interneuron had a small cell body in the fused first abdominal ganglion and extensive longitudinal arborizations. The axon arose from the middle of the neuropile segment and ascended in the medial portion of the connective. **C** Graph showing the decrease in the flight frequency in response to injection of depolarizing current into the interneuron. Increased amount of injected current resulted in greater reduction of flight frequency up to a maximum of 3 nA injected current at which point flight activity was abolished

tion of the connective contralateral to the cell body (Fig. 11B). When depolarized, this interneuron caused a significant reduction in the flight frequency (Fig. 11C). Currents larger than 3 nA completely abolished the flight activity. We observed this effect in all 3 preparations in which this interneuron was penetrated. Experiments to test for the ability of this interneuron to reset the flight rhythm have not yet been performed. In all three preparations there were no oscillations in membrane potential in the absence of the generation of the flight activity as indicated by the dorsal longitudinal EMG. This was also true for all other interneurons we penetrated in this investigation.

Discussion

In this paper we have described a dissected preparation in *L. migratoria* in which the flight rhythm can be easily evoked while recording intracellularly from interneurons and motoneurons in thoracic ganglia. This preparation appears ideally suited for the study of the mechanisms for the patterning of flight motor activity. Already a number of initial observations have advanced our knowledge of the locust flight system as well as providing further data to support some previous proposals.

First, the preparation readily allowed the demonstration in *L. migratoria* of central patterning of flight activity. Rhythmic oscillations in the membrane potential of flight motoneurons occurred following complete deafferentation of the thoracic nerve cord (Fig. 5), and the characteristics of this rhythmic activity were similar to those seen in preparations with less extensive deafferentation. The main effect of deafferentation was to reduce the flight frequency by approximately 50%. A similar reduction in flight frequency following deafferentation has been reported in *S. gregaria* (Wilson 1961). Second, intracellular recordings in flight motoneurons have shown that both excitatory and inhibitory synaptic inputs are important in shaping the form of the membrane oscillations (Fig. 6). In previous studies on the locust flight system (Burrows 1975a, b) it has been difficult to resolve the various components of synaptic input because recordings were made from somata and not from the main neuropile processes as was done in this study. Our recordings from the processes of motoneurons also revealed that the amplitude of oscillations in membrane potential were up to 25 mV which is considerably larger than previously suspected from soma recordings. Third, we identified numerous interneurons whose activity was rhythmically modulated during flight and showed that some of these interneurons made short-latency connections to flight motoneurons (Figs. 8 and 10). Until now no thoracic interneurons have been identified in the flight system of the locust. Finally, we have identified for the first time an interneuron which, when depolarized by current injection, significantly affected the flight frequency, in this case decreasing it (Fig. 11). There are a number of important points which follow from these observations.

The fact that the flight frequency can be easily altered by perturbing the activity of a single interneuron indicates that at least some individual interneurons play a significant role in the generation of the flight rhythm, and the flight rhythm is not entirely generated by the interaction of large sets of identical interneurons. If other interneurons influencing the flight rhythm can be found then the system generating the rhythm may be amenable to analysis. It should be noted, however, that current injection into many other rhythmically active interneurons had no obvious influence on the flight rhythm. Whether or not any of these interneurons are members of the flight oscillator can not be assessed. It is conceivable that some belong to large classes of interneurons and that this prevented current injection from having any significant effect. Clearly more information is required about the interconnections of these interneurons with each other and with motoneurons before their role in the generation of the flight rhythm can be determined.

An important observation was that there were no signs of oscillation in the membrane potential of the interneuron influencing the flight rhythm at times when there was no flight activity in motoneurons (Fig. 11A). Previously it has been suggested that the flight oscillator might be active at all times (Burrows 1975b). Since the interneuron shown in Fig. 11 is presumably a member of the flight oscillator our findings do not support this suggestion. Furthermore, there was no indication in recordings from other interneurons that the flight oscillator was active in the absence of flight motor activity. Thus we conclude the rhythmicity in the flight oscillator is triggered and maintained by specific inputs to the thoracic ganglia, one important source presumably being wind sensitive interneurons descending from the head.

Activation of flight motoneurons was produced by fairly smooth depolarizations and there was no indication for the occurrence of large unitary EPSPs during the depolarizing phase. Thus it appears that motoneurons are driven by many spiking interneurons each producing small EPSPs and/or by graded release of transmitter from non-spiking interneurons. Our finding of a set of homologous interneurons which were located in the metathoracic and each of the first three fused abdominal ganglia (Fig. 9), some

of which evoked small EPSPs in elevator motoneurons (Fig. 8), supports the first of these possibilities. Taking the amplitude and half-duration of the EPSPs to be 1.5 mV and 20 ms respectively and the discharge rate during a burst to be 200 impulses/s it can be easily shown theoretically that each interneuron would cause a depolarization of approximately 3 mV. Input from four such interneurons would therefore produce a depolarization of 12 mV if the effects of non-linear summation of the EPSPs are neglected. Since this amplitude is a significant fraction of the depolarization seen during normal flight (15 to 20 mV) we conclude that these interneurons must be an important set of excitatory drivers of the elevator motoneurons. We have also identified a set of depressor interneurons with structures similar to this set of elevator interneurons (unpublished observations) suggesting that a corresponding set of interneurons might exist for driving the depressor motoneurons. Although these two sets of interneurons may provide the main excitatory drive to elevator and depressor motoneurons during flight, we have not yet excluded the possible involvement of other interneurons, including non-spiking interneurons.

Our findings of interneurons driving flight motoneurons and influencing the flight frequency provide strong support for the conclusion derived from motoneuronal recordings (Burrows 1977) that the patterning of flight activity occurs primarily at the interneuronal level. The question is therefore raised of what role, if any, do motoneurons have in the generation of the flight rhythm? Although we were unable to demonstrate any effect on the flight rhythm by intracellularly injecting current into single motoneurons it is conceivable that groups of motoneurons function cooperatively in rhythm generation. Delayed excitatory effects produced by antidromic stimulation of sets of motoneurons have been interpreted as indicating an important role of motoneurons in patterning of flight activity (Burrows 1973a). However these results are inconclusive in view of the more recent observation that electrical stimulation of flight muscles used to excite motoneurons antidromically also causes excitation of peripheral receptors (Mohl and Nachtigall 1978). It is quite possible therefore that the delayed excitatory effects observed by Burrows (1973a) were due to afferent input causing a weak transient activation of the flight oscillator. This is consistent with the fact that delayed excitation was observed in only 20% of the animals and these were all animals which either flew spontaneously or were easy to coax into flight. We conclude that at present there are no compelling reasons for believing that motoneurons are involved in patterning flight activity, and it is more likely that the generation and the patterning of the basic flight activity occurs entirely at the interneuronal level.

The challenge now is to determine the interconnections between the flight interneurons and their connections to flight motoneurons, to identify the interneurons receiving input from descending wind sensitive neurons, and to find the pathways mediating modulatory actions from wing proprioceptors. The preparation we have described in this paper appears suitable for meeting this challenge. Moreover it provides an opportunity to study the cellular mechanisms of a rhythm generating system with an apparent complexity exceeding that of other invertebrate systems in which these mechanisms have been studied (Getting et al. 1980; Selverston et al. 1976).

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