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CONNECTIONS OF HINDWING TEGULAE WITH FLIGHT NEURONES IN THE LOCUST, *LOCUSTA MIGRATORIA*

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SUMMARY

1. The connections of afferents from the hindwing tegulae to flight motoneurones and interneurones in the locust, *Locusta migratoria*, have been determined by selectively stimulating the tegula afferents while recording intracellularly from identified neurones in the meso- and metathoracic ganglia.

2. Electrical stimulation of the hindwing tegula nerve (nerve 1C1a) revealed two groups of afferents distinguished by a difference in their conduction velocities. Both groups of afferents made excitatory connections to hindwing elevator motoneurones in the ipsilateral half of the metathoracic ganglion. Latency measurements indicated that these connections were monosynaptic. Stimulation of the hindwing tegula nerve also evoked excitatory postsynaptic potentials (EPSPs) in elevator motoneurones in the mesothoracic ganglion and in the contralateral half of the metathoracic ganglion, and inhibitory postsynaptic potentials (IPSPs) in forewing and hindwing depressor motoneurones. The latencies of these evoked EPSPs and IPSPs indicated that the initial responses were produced *via* interneuronal pathways.

3. None of the recordings revealed EPSPs in depressor motoneurones or IPSPs in elevator motoneurones in response to hindwing tegula stimulation. This observation differs from that in *Schistocerca gregaria* where it has been reported that the large tegula afferents produce EPSPs in depressors and IPSPs in elevators (Kien & Altman, 1979).

4. Some of the interneurones in disynaptic excitatory and inhibitory pathways to motoneurones were identified. These interneurones received input from both hindwing tegulae and were readily excited beyond threshold by mechanical stimulation of the tegulae or by electrical stimulation of the tegula afferents. The contribution of one excitatory interneurone to the electrically evoked EPSPs was assessed by blocking spike initiation in the interneurone while recording simultaneously from a flight motoneurone.

5. Based on our observations of the central connections of tegula afferents to flight motoneurones and the previously reported discharge patterns of these afferents during tethered flight (Neumann, 1985), we propose that a major function of the hindwing tegulae in *L. migratoria* is to generate the initial depolarizations in forewing and hindwing elevator motoneurones during flight. Consistent with this

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proposal was our finding that ablation of the hindwing tegulae delayed the onset of elevator activity relative to the onset of the preceding depressor activity.

INTRODUCTION

Over the past decade there has been an increasing interest in establishing the properties, central connections and functions of the various groups of proprioceptors associated with the wings in the locust. This interest has arisen from the recognition that sensory feedback plays an important part in patterning motor activity for flight (Wendler, 1974, 1983; Altman, 1983). Phasic sensory signals have been shown to determine precisely the timing of some aspects of the flight motor pattern (Möhl, 1985; Neumann, 1985; Bacon & Möhl, 1983; Horsmann, Heinzell & Wendler, 1983; Wolf & Pearson, 1987) and to participate in the generation of the flight rhythm (Horsmann & Wendler, 1985; Pearson, Reye & Robertson, 1983). A major task now is to establish the cellular basis for the effects that the different groups of proprioceptors have on the flight motor pattern. A necessary step towards this goal is to determine the connections made by sensory afferents to flight motoneurones and interneurones. We now know some of the central connections made by afferents from the wing stretch receptors (Burrows, 1975; Reye & Pearson, 1987), the wing companiform sensillae (Elson, 1987; Horsmann & Wendler, 1985) and the tegulae (Kien & Altman, 1979).

The tegulae are complex sensory organs located near the anterior base of each wing. Each of these knob-like structures is composed of a field of mechanosensory hairs (about 40) and a chordotonal organ (Kutsch, Hanloser & Reinecke, 1980). About 80 afferents arise from each tegula, and these range in diameter from about 1 to 10 μm (Altman, Anselment & Kutsch, 1978). The afferents from both groups of receptors are excited during the downstroke of the wing, with the onset of their discharge preceding the onset of elevator activity (Neumann, 1985). The function of the phasic sensory information provided by the tegulae has not yet been established. In the only previous study concerned with the connections of tegula afferents with central neurones, Kien & Altman (1979) reported for *Schistocerca gregaria* that two groups of afferent fibres arising from the hindwing tegulae produced opposite effects in hindwing motoneurones. The larger, fast-conducting group produced EPSPs in depressor motoneurones and IPSPs in elevator motoneurones, whereas the smaller, slow-conducting group produced EPSPs in elevators and IPSPs in depressors. Which receptors are associated with these two groups of afferents has not yet been established.

An aim in our present studies on the flight system of the locust has been to determine the function of the wing tegulae. In preliminary experiments on *Locusta migratoria* we observed that direct electrical stimulation of the hindwing tegula nerve (nerve 1C1a) always evoked EPSPs in elevator motoneurones and IPSPs in depressor motoneurones. Since these results differed from those reported for *S. gregaria* by Kien & Altman (1979) we decided to investigate in detail the central connections of the hindwing tegula afferents in *L. migratoria*. In this paper we report the results of

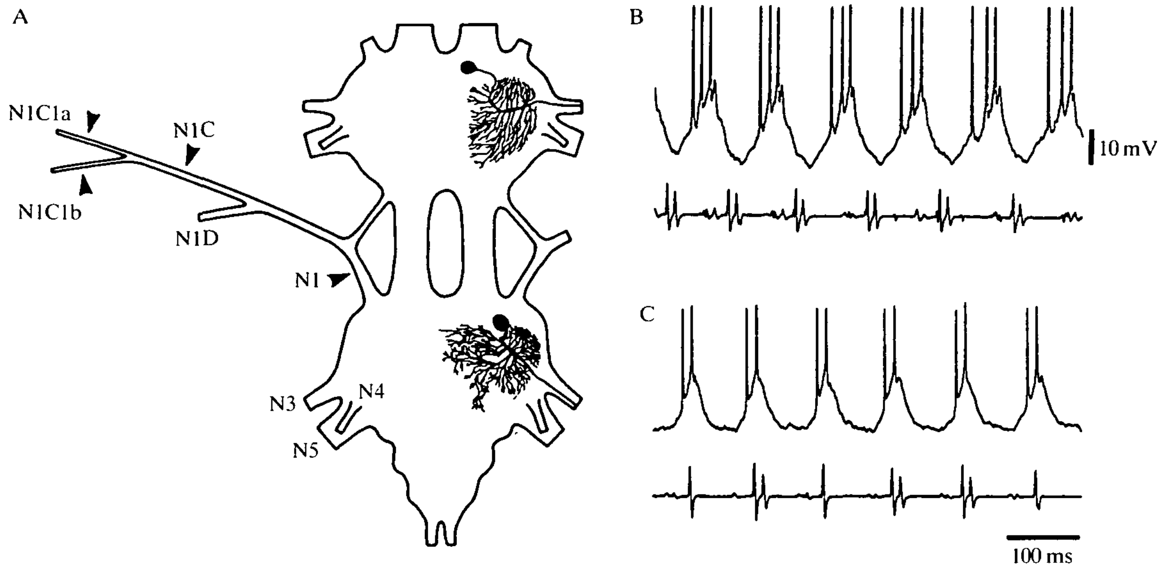

Fig. 1. (A) Diagram of the meso- and metathoracic ganglia showing the stimulating and recording sites on metathoracic nerve 1 (N1) and its branches used in this investigation (see text for details). Drawings of two flight motoneurones are shown in this diagram; an anterior tergocoxal motoneurone (elevator) in the mesothoracic ganglion (top) and the first basalar motoneurone (depressor) in the metathoracic ganglion (bottom). Intracellular recordings from these motoneurones during wind-induced rhythmic activity are shown in B and C, respectively. The timing of the depolarizations in flight motoneurones was determined with respect to the EMG activity recorded from one hindwing subalar (depressor) muscle (large spike in lower traces of B and C).

this investigation. In addition, we describe some of the interneurones involved in transmitting information from the hindwing tegulae to flight motoneurones and the effect of hindwing tegula ablation on some aspects of the flight motor pattern.

MATERIALS AND METHODS

Animals

All experiments were performed at room temperature (approx. 23 °C) on adult locusts, *Locusta migratoria* (L.), obtained from a laboratory culture at the University of Alberta. Male and female animals were used and no differences were noted with respect to sex.

Preparation for determining the connections of tegula afferents

To determine the central connections of hindwing tegula afferents a preparation was used which allowed intracellular recordings to be made from neurones in the meso- and metathoracic ganglia while stimulating electrically the nerve branches arising from either one or both of the two hindwing tegulae (N1C1a, Fig. 1).

Animals were mounted dorsal side up on a cork board and the thoracic ganglia were exposed following a dorsal midline incision and the removal of the gut and the muscles lying dorsal to the ganglia. The meso- and metathoracic ganglia were lifted from the ventral cuticle and mounted on a rigid stainless steel plate. The lateral nerves 1, 3 and 4 of the mesothoracic ganglion and nerves 3 of the metathoracic ganglion were severed on both sides to reduce movements of the ganglia during rhythmic motor activity. Rhythmic motor activity in flight motoneurones was induced by a wind stream on the head (Robertson & Pearson, 1982) and monitored by recording electrodes in a hindwing depressor muscle, the subalar muscle 129. The branches of nerve 1 supplying the hindwing itself (nerve 1C1b) and the hindwing tegula (nerve 1C1a) were exposed on both sides by removing the dorsal longitudinal muscles and the elevator muscles (muscles 113 and 118) lying adjacent to nerve 1C. The hindwing tegulae were exposed to allow them to be stimulated by a hand-held probe. Extracellular hook electrodes for either recording or stimulating were placed at various locations on nerve 1 and its branches (see Fig. 1). Which sites were chosen depended on the particular experiment (details are given in the Results).

Extracellular hook electrodes were made of silver wires 75 μm in diameter. These were used in either a bipolar configuration or a monopolar configuration with the indifferent electrode placed nearby in muscle or haemolymph but not close to any branches of nerve 1. The section of the nerve which had been placed on the electrode(s) was coated with petroleum jelly. The effectiveness of the insulation was monitored by noting the large increase in the amplitude of spontaneous afferent spikes following the application of the petroleum jelly.

Standard techniques were used to record intracellularly from flight motoneurones and interneurones in the meso- and metathoracic ganglia (see Robertson & Pearson, 1982, for details). Electrodes were filled with a 5% solution of Lucifer Yellow. Following the recording of data Lucifer Yellow was injected into the cell by the application of a constant 2–5 nA negative current for 2–5 min. The ganglia were removed from the animal, fixed in a 4% solution of paraformaldehyde for 30 min, dehydrated and cleared in methyl salicylate. The stained neurones were viewed as wholemounts using an epifluorescence microscope.

Two criteria were used to identify neurones from which intracellular recordings were made. The first was their structure, as determined by the intracellular injection of Lucifer Yellow, and the second was their pattern of activity during rhythmic sequences induced by wind on the head. The classification of a motoneurone as either an elevator or a depressor was made by noting the timing of the rhythmic depolarizations relative to the electromyographic (EMG) activity recorded from the hindwing subalar muscle (Fig. 1B,C). A previous study (Hedwig & Pearson, 1984) gives the details of the structural and physiological properties that allowed the unambiguous identification of motoneurones supplying the main flight muscles. Two interneurones (504 and 511) examined in this study have been described in previous publications (Robertson & Pearson, 1983, 1985). We describe two other interneurones (515 and 566) for the first time. The properties of these interneurones are described in the Results.

Electromyographic recording of flight motor activity

To obtain information on the effects of hindwing tegulae ablation on the flight pattern, electromyographic recordings were made from flight muscles in tethered flying animals. The activity patterns were compared in intact animals and in animals following surgical removal of the hindwing tegulae. The hindwing tegulae were surgically removed by transecting their bases with a pair of fine scissors. Extracellular recordings from metathoracic nerve 1 showed that this procedure did not damage afferents in the nearby nerve 1C1b. Moreover, no afferent activity could be evoked by probing the regions of the cuticle close to the ablated tegulae. This observation indicated the complete removal of input from the tegulae.

The procedure for recording the EMG patterns was similar to that described by Pearson & Wolf (1987). Animals were mounted about 10 cm in front of a wind tunnel and the EMG patterns were measured during flight activity induced by directing a continuous air stream (velocity between 2 and 3 $m s^{-1}$) towards the animal. The muscles chosen for study were the tergosternal muscles (wing elevators) and first basalar muscles (wing depressors) of the forewings and hindwings. These muscles are pure flight muscles, they receive innervation from only a single fast motoneurone, and they are readily accessible for EMG recording. The spike activity recorded from individual muscles was fed into a trigger circuit which produced output pulses corresponding to the negative peaks in the EMG recordings. The output pulses from the trigger circuit were then fed to an LSI 11/23 computer for analysis. Data collection commenced about 5 s after the initiation of flight activity, and individual trials lasted 30–60 s.

RESULTS

When examining the central connections of any group of receptors it is essential that reliable criteria are established for the selective activation of the afferents from this group of receptors. In the first section of Results we describe the procedures we used for selective activation of afferents from the hindwing tegulae. The connections of these afferents to motoneurones and interneurones are described in the subsequent sections.

Selective stimulation of tegula afferents

Electrical stimulation

The most direct method to activate electrically tegula afferents was to stimulate the fine nerve (nerve 1C1a) arising from the tegula (Fig. 2A). This nerve is sufficiently long (about 1 mm) to allow the placement of either single or paired stimulating electrodes. A potential problem with this method of stimulation is that current spread might excite afferents in the nearby wing nerve (nerve 1C1b). To investigate whether this occurred at the stimulus voltages used in the present study, we compared the potentials recorded from the nerve root 1 close to the ganglion in

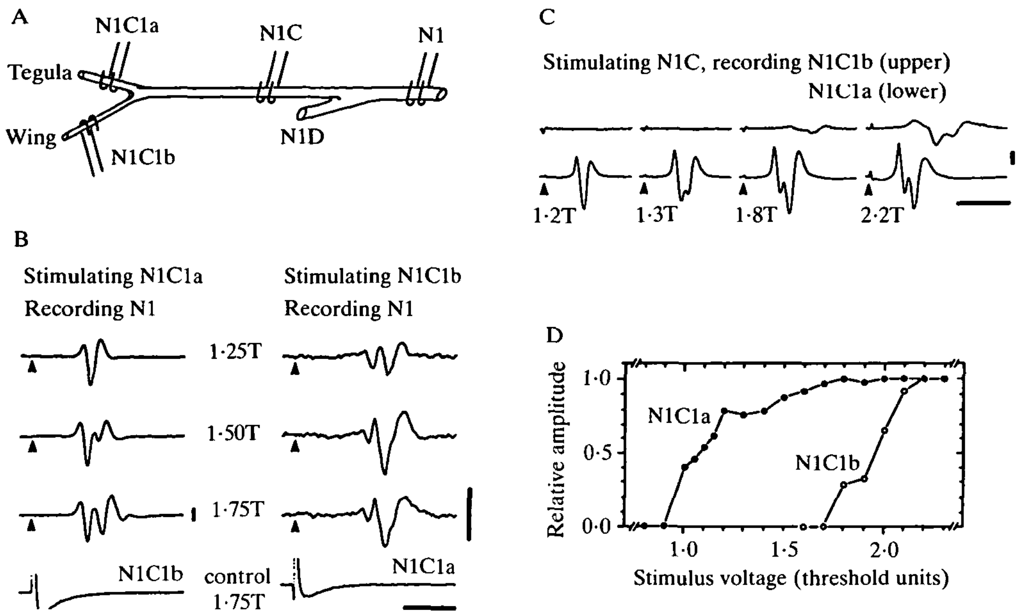

Fig. 2. Selective stimulation of afferents from the hindwing tegulae. (A) Diagram showing the stimulating and recording sites on metathoracic nerve 1 (N1) and its branches. (B) Extracellularly recorded potentials from N1 in response to stimulating either the tegula nerve N1C1a (left set of records) or the wing nerve N1C1b (right set of records). The records show the potentials at stimulus strengths 1·25, 1·5 and 1·75 times the threshold voltage (T) necessary for evoking the first detectable response in the N1 recording. The bottom traces show that at 1·75 times threshold the stimulus delivered to N1C1a did not spread to excite afferents in N1C1b and *vice versa*. Note the longer latencies and smaller amplitudes of the potentials evoked by N1C1b stimulation. (C) Extracellularly recorded potentials from the tegula (N1C1a) and wing (N1C1b) nerves in response to increasing the stimulus voltage applied to N1C. Note the lower threshold for activating afferents in the tegula nerve. The peak to peak amplitudes of the evoked potentials in the two nerves are plotted in D as a function of stimulus intensity. The plots show the large difference in threshold for activating tegula and wing nerve afferents. Note that almost all the tegula afferents are excited at the threshold for wing afferents. The arrowheads in B and C indicate the times of stimulus application. Calibrations: vertical 1 mV, horizontal 2·5 ms.

response to stimulation of either the tegula nerve, N1C1a, or the wing nerve, N1C1b (Fig. 2B).

The potentials recorded from nerve 1 in response to stimulation of the tegula nerve showed either one or two components depending on the stimulus strength (Figs 2B, 3). At stimulus voltages just above threshold for the largest afferents the response recorded from nerve root 1 using a single recording electrode had a triphasic waveform (Fig. 3). A triphasic waveform that varies in amplitude depending on stimulus strength is to be expected if a single group of afferents with a fairly narrow distribution of diameters was being stimulated (Stein & Pearson, 1971). When the

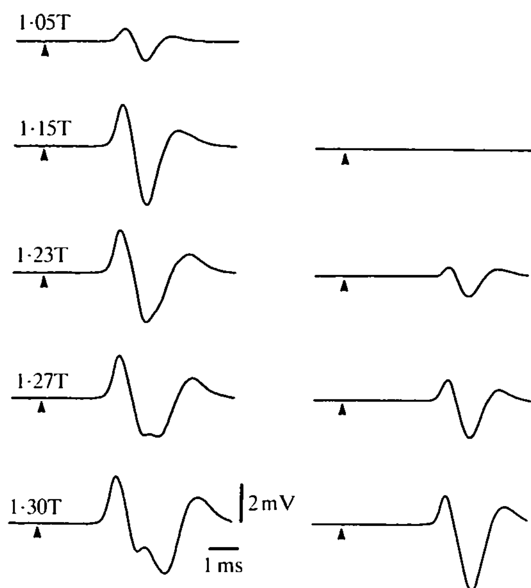

Fig. 3. Records showing that afferents arising from the hindwing tegulae can be divided into two groups according to stimulus threshold and conduction velocity. The traces on the left show the potentials recorded extracellularly from nerve 1 in response to increasing the stimulus strength to the tegula nerve (N1C1a). At stimulus strengths at or below 1·15 times threshold (T) a triphasic potential was recorded whose amplitude increased with stimulus strength. The amplitude of this potential was maximal at 1·15T. A second triphasic potential was evoked and superimposed on the first when the stimulus strength was increased beyond 1·15T. The second potential could be visualized by subtracting the maximal first component, i.e. the response at 1·15T, from the responses recorded at higher stimulus strengths. The traces on the right show the results of this subtraction. The arrowheads indicate the times of stimulation.

stimulus strength was increased, a second, slower-conducting group of afferents was recruited (Fig. 3). The clear distinction between the two groups of afferents was revealed by the distinct second negative peak in the potential recorded from nerve root 1, and by a second triphasic waveform which resulted from subtracting the maximal first triphasic wave from the recording (Fig. 3, right). The mean conduction velocities of the two groups of afferents were about 2·6 and 2·2 $m\,s^{-1}$.

The potentials recorded from nerve root 1 in response to stimulation of the wing nerve (N1C1b) (Fig. 2B, right) differed in two major aspects from those evoked by tegula nerve stimulation. First, the maximum amplitudes of the potentials were about four times smaller and, second, the maximum conduction velocity was less than that of the slower component resulting from tegula nerve stimulation. The distinctly longer conduction time of wing nerve afferents allowed us to verify that tegula nerve stimulation did not excite wing nerve afferents by current spread. Even with voltages 1·75 times the threshold value for activating the largest tegula afferents there was no sign of a small late component that could have corresponded to the

activation of wing nerve afferents (Fig. 2B, left). Since the maximal stimulus strengths used in our experiments never exceeded 1·5 times the threshold voltage for the large afferents, we conclude that we selectively activated tegula afferents. The validity of this conclusion was supported by the observation that stimuli delivered to the tegula nerve which maximally activated both groups of tegula afferents did not evoke potentials in the wing nerve (Fig. 2B, bottom left).

The significantly lower conduction velocity of wing nerve afferents relative to the conduction velocity of tegula afferents indicated that there exists a wide separation of fibre diameters of afferents in tegula and wing nerves. This has been reported in anatomical studies (Altman *et al.* 1978). One would expect, therefore, that the threshold for electrical stimulation of tegula afferents in nerve 1C would be lower than that for stimulation of wing nerve afferents in the same nerve. To demonstrate this we simultaneously recorded the antidromic potentials in the tegula and the wing nerves elicited by stimulation of nerve 1C (Fig. 2C). This experiment revealed a clear separation of the threshold voltages for activating the two groups of afferents. Wing nerve afferents were not excited until the stimulus strength was raised to about 1·8 times the threshold for the largest tegula afferents. Usually the threshold voltage for activating the wing nerve afferents was close to the voltage necessary for giving a maximal response in the tegula nerve (Fig. 2D). These observations led us to conclude that by careful adjustment of the stimulus strength a selective activation of the tegula afferents could also be achieved by electrical stimulation of nerve 1C. This method of stimulating tegula afferents was used in some of our experiments. However, because there was a risk of co-activating wing nerve afferents, we verified results obtained with this method by mechanically stimulating the tegulae.

Mechanical stimulation

Selective activation of tegula afferents could also be achieved by gently touching the tegulae with a hand-held probe. When using this method we usually cut the wing nerve 1C1b to eliminate the possibility of activating wing receptors. However, this was not essential because gentle probing in areas immediately adjacent to the tegulae failed to elicit any responses in afferents of nerve 1 when nerve 1C1b was intact. Apart from its selectivity, the major advantage of mechanical stimulation of the tegulae was that it allowed the projection of single afferents to be determined by noting the 1:1 correspondence of spikes recorded from nerve 1C in response to tegula stimulation and unitary EPSPs in central neurones.

Connections of hindwing tegula afferents to flight motoneurones

EPSPs in elevators

Electrical stimulation of the hindwing tegula nerve (N1C1a) evoked an EPSP in ipsilateral elevator motoneurones of the metathoracic ganglion (Figs 4, 5). By monitoring the afferent input in recordings from nerve root 1 we found that both groups of tegula afferents excited these motoneurones. At low stimulus strengths, which excited only the largest tegula afferents, an EPSP was evoked in every elevator

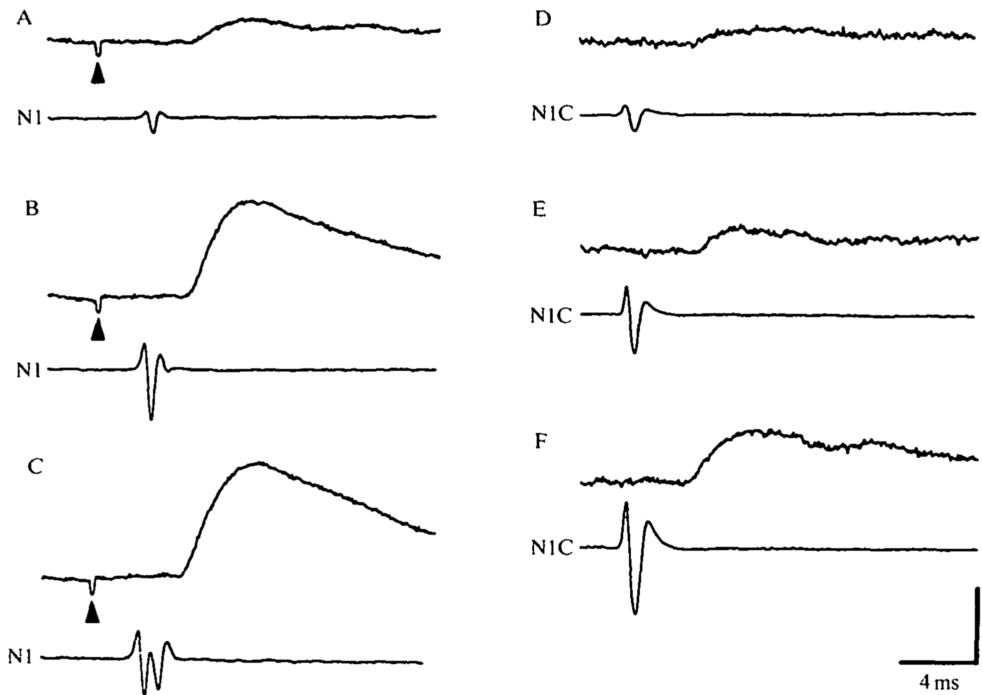

Fig. 4. Excitatory connections of hindwing tegula afferents to elevator motoneurones in the ipsilateral metathoracic ganglion. (A–C) Compound EPSPs recorded intracellularly in an anterior tergocoxal motoneurone (top traces) in response to electrical stimulation of the ipsilateral tegula nerve (N1C1a). The stimulus artefacts are indicated by arrowheads. The afferent volley from the tegula nerve stimulation was monitored by an extracellular recording electrode on nerve 1 (N1). (A) At stimulus strengths just above threshold for activating tegula afferents an EPSP was recorded in the motoneurone. (B) Stimulation of only the group of large tegula afferents at a higher stimulus strength increased the amplitude of the EPSP. (C) Additional recruitment of the group of smaller tegula afferents at a still higher stimulus strength further increased the amplitude of the EPSP. (D–F) Unitary EPSPs recorded intracellularly from a tergosternal motoneurone (top traces) in response to single afferents arising from the ipsilateral hindwing tegula. The spikes from the single tegula afferents were recorded from nerve 1C (bottom traces) and these spikes were used to trigger the oscilloscope sweeps. Note the correlation between the amplitudes of the unitary EPSPs and the size of the tegula afferent (indicated by the amplitude of the extracellularly recorded spike). Calibrations: vertical (intracellular recordings) A–C 5 mV, D–F 2 mV.

motoneurone from which we recorded (Fig. 4A,B). Increasing the stimulus strength to recruit the more slowly conducting group of tegula afferents led to an increase in the amplitude of the EPSP (Figs 4C, 5). That this increase in amplitude was due to recruitment of the smaller tegula afferents and not to recruitment of additional large afferents was indicated by the observation that the onset of the increase in the EPSP amplitude was delayed with respect to the onset of the EPSP itself by an amount close to the difference in conduction time for the large and small afferents (Fig. 5).

In more than 100 recordings from hindwing elevator motoneurones (which included recordings from members of all the four main groups of elevator motoneurones) we never observed IPSPs in response to stimulation of either the large or the small tegula afferents in the ipsilateral tegula nerve.

The latency of the EPSPs evoked in the ipsilateral metathoracic elevators was about 4 ms with respect to the stimulus. The conduction time from the point of stimulation to the metathoracic ganglion accounted for the major part of this latency. To estimate the central synaptic delay we penetrated the axon of a tegula afferent in the metathoracic ganglion and observed that the time difference between the peak of this afferent spike and the onset of the EPSPs in elevator motoneurones was 0·8–1·2 ms. This latency is consistent with the existence of a monosynaptic connection between the tegula afferents and the ipsilateral metathoracic elevator motoneurones. Further evidence for a direct monosynaptic connection was the

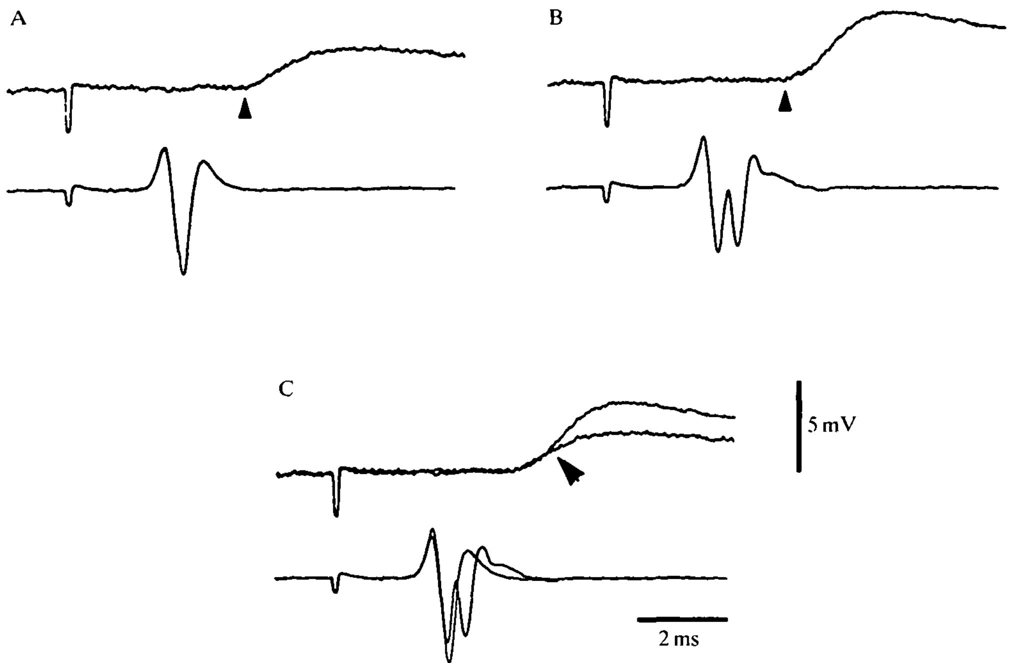

Fig. 5. Records showing that both the larger, fast-conducting group and the smaller, slower-conducting group of tegula afferents make excitatory connections to ipsilateral hindwing elevator motoneurones. Top traces: intracellular recordings from an anterior tergocoxal motoneurone. Bottom traces: extracellular recordings from nerve 1. (A,B) Compound EPSPs recorded in the motoneurone in response to electrical stimulation of the ipsilateral hindwing tegula nerve (N1C1a). The initial negative deflection indicates the time of stimulus presentation and the triangles mark the time of onset of the EPSPs. In A only the larger, fast-conducting tegula afferents were stimulated, whereas in B both groups of tegula afferents were stimulated. (C) Superposition of the two sets of records shown in A and B. Note that the onset of the additional depolarization produced by the recruitment of the group of smaller afferents (onset marked by arrowhead) was delayed by an amount corresponding to the longer conduction time of the smaller afferents.

occurrence of unitary EPSPs in elevator motoneurones following each spike in tegula afferents (Fig. 4D–F). Finally, the anatomical organization is consistent with monosynaptic connections since the central projections of the tegula afferents overlap the processes of elevator motoneurones in the dorsal neuropile (Tyrer & Altman, 1974).

Stimulation of hindwing tegula afferents also evoked EPSPs in contralateral metathoracic elevator motoneurones and in elevator motoneurones on both sides of the mesothoracic ganglion (Fig. 6). These EPSPs differed in two respects from those observed in ipsilateral metathoracic elevators: (i) the onset latency was about 3 ms longer (i.e. about 7 ms), and (ii) multiple late components often occurred in the EPSPs. The longer latencies and the multiple components of these EPSPs suggested that they were evoked *via* a pathway containing at least one interneurone. This was to be expected since the central projections of the hindwing tegula afferents are confined to the hemiganglion which they enter (Kien & Altman, 1979). The slightly longer latency of the EPSPs in the mesothoracic elevators than in the contralateral elevators (approx. 1 ms) can be ascribed to the conduction time in the axons of ascending interneurones. Although the pathways from the hindwing tegulae to mesothoracic and to contralateral metathoracic elevators are indirect they are quite powerful. For example, mechanical stimulation of one hindwing tegula usually produced suprathreshold depolarizations in these elevator motoneurones (Fig. 6B).

Again, as with the tegula projection to ipsilateral metathoracic elevators, there was no indication that tegula afferents could produce inhibitory potentials in mesothoracic and contralateral metathoracic elevator motoneurones. In all our recordings from elevator motoneurones the response to tegula nerve stimulation was always an EPSP. As the stimulus voltages were increased the EPSP amplitudes increased but no signs of IPSPs were observed.

IPSPs in depressors

Electrical stimulation of either one of the hindwing tegula nerves evoked IPSPs in wing depressor motoneurones (Fig. 7). The amplitudes of the evoked IPSPs ranged from 1 to 5 mV and often a number of discrete components could be discerned in the compound IPSP. The latency of the evoked IPSPs was 6–8 ms for all depressor motoneurones. For the depressor motoneurones in the metathoracic ganglion ipsilateral to the stimulated tegula nerve this latency was about 3 ms longer than for the EPSPs evoked in the elevator motoneurones of the same hemiganglion. The onset of the IPSPs followed the spikes recorded centrally from tegula afferents after about 4 ms. These latencies indicated that all the IPSPs observed in depressor motoneurones in response to hindwing tegula stimulation were produced *via* interneuronal pathways.

Although the inhibition of depressor motoneurones by tegula input was not monosynaptic, tegula activity did have a noticeable inhibitory effect on tonic depressor activity. When one hindwing tegula was mechanically stimulated it caused suppression of ongoing activity in the first basalar motoneurone in the contralateral mesothoracic ganglion (Fig. 7B).

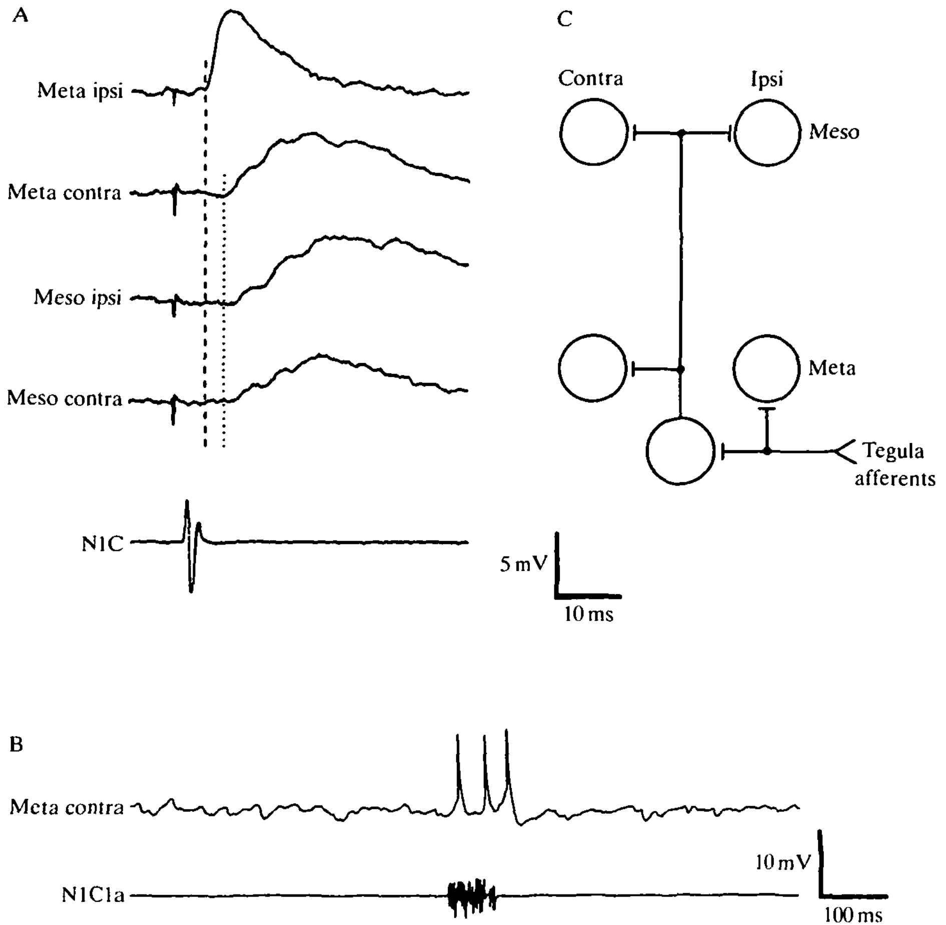

Fig. 6. Excitatory connections of hindwing tegulae to hindwing and forewing elevator motoneurones. (A) Compound EPSPs evoked in elevator motoneurones in response to electrical stimulation of one hindwing tegula nerve (N1C1a). The stimulus strength was adjusted to activate the group of large tegula afferents but not the group of smaller afferents. The afferent volley was monitored by recording from nerve 1C (bottom trace). The initial negative deflection in each record indicates the time of stimulation. The recordings were made sequentially from each motoneurone: meta ipsi, metathoracic tergosternal ipsilateral to the stimulated nerve; meta contra, metathoracic tergosternal contralateral to the stimulated nerve; meso ipsi, mesothoracic tergosternal ipsilateral to the stimulated nerve; meso contra, mesothoracic anterior tergocoxal contralateral to the stimulated nerve. The dashed and dotted lines indicate the times of onset of the EPSPs in the ipsilateral metathoracic elevator and contralateral metathoracic elevator, respectively. Note that the onset of the EPSPs in the ipsilateral metathoracic elevator precedes the onset of the EPSPs in the other elevators by 2–3 ms. (B) Mechanical stimulation of one hindwing tegula evoked spike activity in an elevator motoneurone in the contralateral half of the metathoracic ganglion. Afferent activity was monitored by recording from the tegula nerve (N1C1a). (C) Schematic diagram showing the excitatory connections (bars) of the hindwing tegula afferents to elevator motoneurones in the meso- and metathoracic ganglia. The latencies of the EPSPs in forewing and contralateral hindwing elevators indicate the existence of at least one interneurone in the pathway from hindwing tegula afferents to these motoneurones.

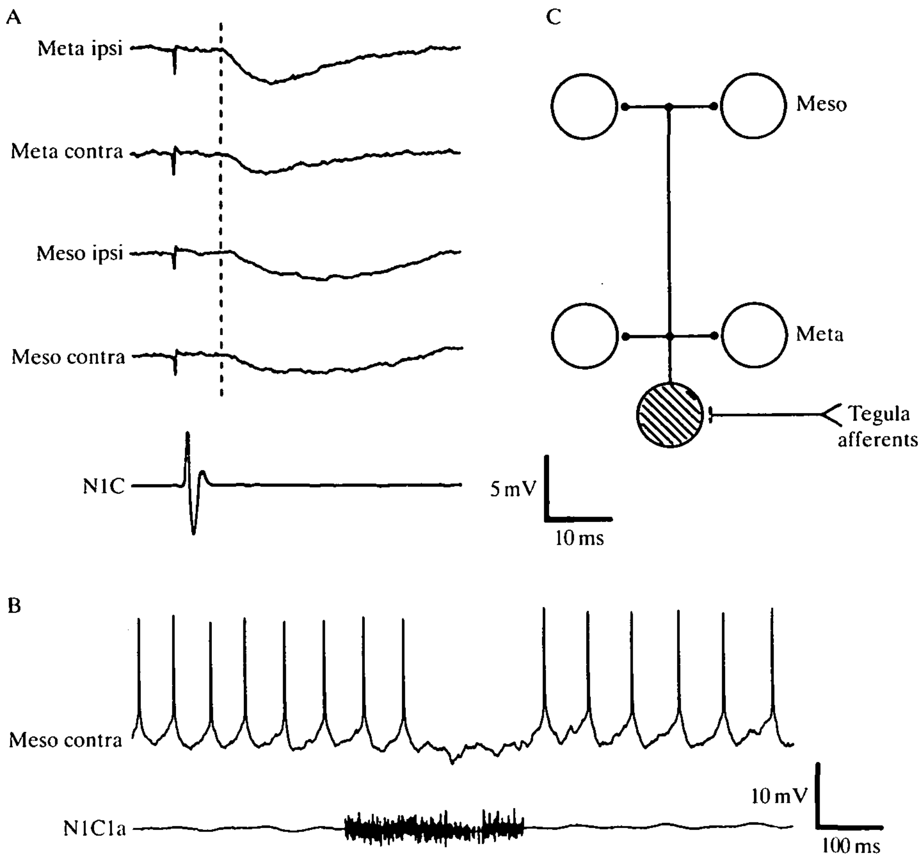

Fig. 7. Inhibitory connections of hindwing tegulae to forewing and hindwing depressor motoneurones. (A) IPSPs evoked in depressor motoneurones in response to electrical stimulation of one hindwing tegula nerve (N1C1a). The stimulus strength was adjusted to activate only the group of large tegula afferents. The afferent volley was monitored by recording from nerve 1C (bottom trace). The initial negative deflection in each record indicates the time of stimulation. The recordings were made sequentially from each motoneurone: meta ipsi, metathoracic first basalar ipsilateral to the stimulated nerve; meta contra, metathoracic second basalar contralateral to the stimulated nerve; meso ipsi, mesothoracic first basalar ipsilateral to the stimulated nerve; meso contra, mesothoracic first basalar contralateral to the stimulated nerve. The dashed line indicates the time of onset of the IPSP in the ipsilateral metathoracic depressor. Note that the latencies of the IPSPs in other depressors were slightly longer. (B) Mechanical stimulation of one hindwing tegula inhibits spontaneous spike activity in a depressor motoneurone in the contralateral half of the mesothoracic ganglion. Afferent activity was monitored by recording from the tegula nerve (nerve 1C1a). (C) Schematic diagram showing the inhibitory connections of the hindwing tegula afferents to depressor motoneurones in the meso- and metathoracic ganglia. The latencies of the IPSPs indicate that all connections are *via* interneuronal pathways. Tegula afferents excite (bar) at least one inhibitory interneurone (crosshatched). This interneurone is shown inhibiting depressor motoneurones on both sides of the meso- and metathoracic ganglia.

In over 100 recordings from depressor motoneurones in the meso- and metathoracic ganglia (this included recordings from members of all groups of depressor motoneurones) we never observed EPSPs in response to selective stimulation of tegula afferents. IPSPs could be evoked when only the larger tegula afferents were excited and the IPSP amplitude usually increased with the additional recruitment of the smaller group of tegula afferents at higher stimulus voltages.

Interneurones in the pathway from tegula afferents to flight motoneurones

Figs 6C and 7C summarize the connections we have observed from the hindwing tegulae to flight motoneurones in the meso- and metathoracic ganglia. All the elevator motoneurones we examined were excited by tegula input, with the ipsilateral metathoracic elevators receiving a direct monosynaptic connection and the remainder being excited *via* interneuronal pathways. All the depressor motoneurones we examined were inhibited by tegula input *via* pathways containing at least one interneurone. Having established the synaptic influences of hindwing tegula input to flight motoneurones, our next aim was to identify some of the interneurones mediating the excitatory and inhibitory inputs to elevator and depressor motoneurones, respectively.

Excitatory interneurones

In this study we identified an interneurone in the metathoracic ganglion that monosynaptically excited elevator motoneurones in both the meso- and the metathoracic ganglia and received strong excitatory input from tegula afferents. We have labelled this interneurone 566 (Fig. 8A). Its soma is located close to the dorsal surface in the posterior lateral region of the metathoracic ganglion and its main neurite crosses the midline about 100 μm below the dorsal surface in dorsal commissure VI of the metathoracic neuromere. The axon of 566 joins the lateral dorsal tract and projects anteriorly in the lateral region of the meso–metathoracic connective. Processes arising from the axon project into the dorsolateral neuropile and into the fused abdominal ganglia. During wind-induced rhythmic activity in deafferented preparations the oscillations in membrane potential were weak (Fig. 8B). Usually only one spike (or at the most two) was generated late in the interval between consecutive depressor spikes. Often the membrane potential oscillations remained subthreshold (Fig. 8B). A striking property of the 566 interneurones was that they produced large unitary EPSPs in forewing elevator motoneurones (Fig. 8C). These EPSPs were usually 4–6 mV in amplitude. The amplitudes of unitary EPSPs from other flight interneurones to motoneurones have been found to be 0·1–2 mV (Robertson & Pearson, 1983, 1985). The latency of each EPSP following a spike in 566 was about 2 ms. This indicated that interneurone 566 makes monosynaptic connections to forewing elevator motoneurones.

Interneurone 566 received a strong excitatory input from both hindwing tegulae (Fig. 9). This was demonstrated by mechanical stimulation of either hindwing tegula (Fig. 9D,E). The connections of tegula afferents to interneurone 566 appeared to be monosynaptic because there was a 1:1 correspondence between spikes recorded

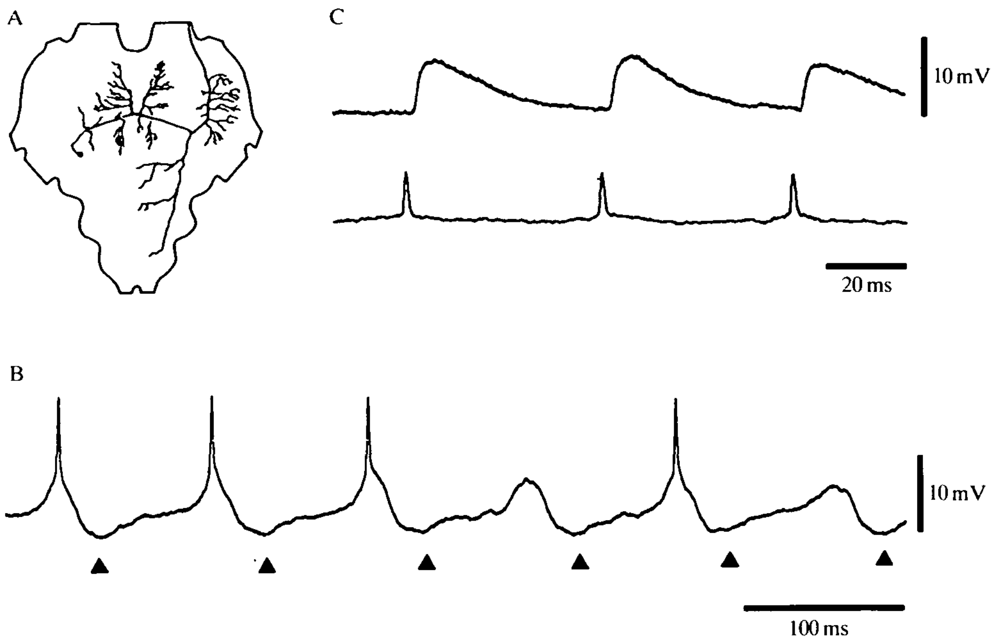

Fig. 8. (A) Drawing of interneurone 566 in the metathoracic ganglion. (B) Oscillations in the membrane potential of interneurone 566 in response to a wind stimulus directed onto the head of a deafferented preparation. The triangles indicate the time of depressor activity. Note that spikes were not generated in each cycle. (C) Excitatory connection from interneurone 566 to an elevator motoneurone in the mesothoracic ganglion. Top trace, intracellular recording from the elevator motoneurone. Bottom trace, intracellular recording from interneurone 566. Note that each spontaneously occurring spike in interneurone 566 was followed by a large EPSP in the elevator motoneurone.

extracellularly from tegula afferents in nerve 1C and unitary EPSPs in the interneurone (Fig. 9A–C), and the latencies of the EPSPs were similar to those in ipsilateral hindwing elevator motoneurones. Interneurone 566 was also strongly depolarized by electrical stimulation of either of the hindwing tegula nerves. Since these depolarizations were suprathreshold at modest stimulus strengths we concluded that 566 contributed to the generation of the EPSPs in forewing elevator motoneurones in response to hindwing tegula stimulation. The extent of this contribution was assessed by hyperpolarizing interneurone 566 to prevent spike initiation. This resulted in a reduction of the amplitude of the evoked EPSP by about 50 % (Fig. 10). The failure of spike block in interneurone 566 to abolish completely the excitatory response in forewing elevator motoneurones indicated that other interneurones form part of the excitatory pathway. We consider it unlikely that there is more than one 566 interneurone projecting to each side of the mesothoracic ganglion because we have not yet observed more than one (not counting the contralateral homologue) following multiple dye injections in a single preparation. A more likely possibility is that interneurones previously labelled as 504 and 514

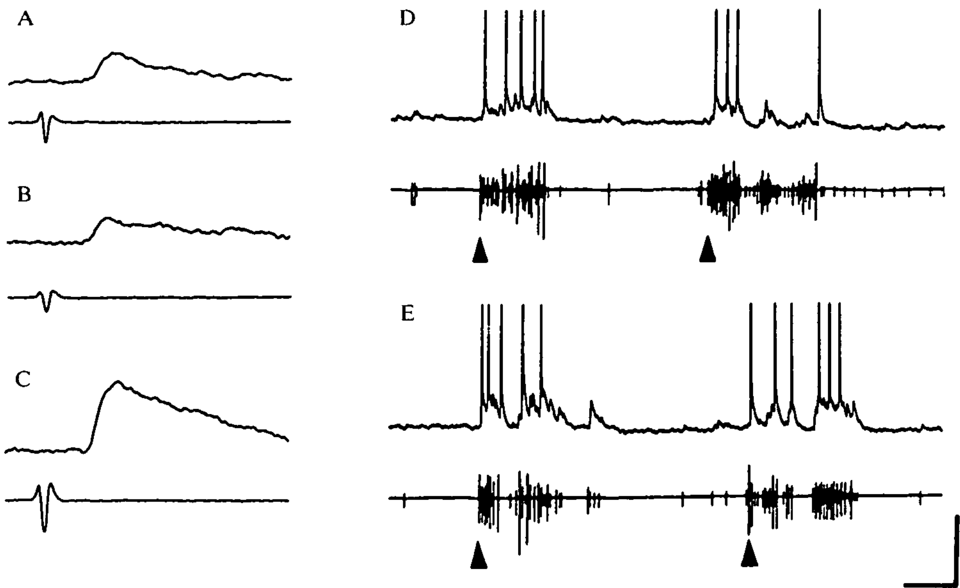

Fig. 9. Excitatory connections of hindwing tegula afferents to interneurone 566. Top traces, intracellular recordings from interneurone 566. Bottom traces, extracellular recordings from nerve 1C. (A–C) Unitary EPSPs evoked in interneurone 566 by single action potentials in tegula afferents. The oscilloscope was triggered from the spikes in the tegula afferents. (A) Unitary EPSP evoked by an afferent located contralateral to the soma of interneurone 566. (B,C) Unitary EPSPs evoked by two afferents located ipsilateral to the soma of interneurone 566. Note that the larger afferent gave rise to a larger unitary EPSP. (D,E) Excitatory input to interneurone 566 in response to touching the tegula contralateral (D) and ipsilateral (E) to the soma of the interneurone. The arrowheads indicate the onset of activity in tegula afferents recorded from nerve 1C each time the tegula was touched. Calibrations: vertical (intracellular recordings) A–C 2 mV, D,E 10 mV; horizontal A–C 4 ms, D,E 150 ms.

(Robertson & Pearson, 1983, 1985) form part of the excitatory pathway. These interneurones make excitatory connections to forewing elevator motoneurones, and we found that both received excitatory input from hindwing tegula afferents. Fig. 11D,E shows excitation of interneurone 504 by electrical and mechanical stimulation of the hindwing tegulae.

Structurally, interneurone 566 resembles the 504 neurones in the fused abdominal ganglia and 566 may be the metathoracic homologue of the 504 neurones, as was assumed in an earlier study (Robertson, Pearson & Reichert, 1982). However, interneurone 566 has a nuxber of physiological properties distinctly different from the 504 neurones, the most notable being the weaker oscillations in membrane potential of 566 and the much stronger excitatory input of 566 to forewing elevator motoneurones. Because of these distinct differences we have chosen to distinguish the 566 interneurones from the 504 interneurones.

The 504, 514 and 566 interneurones all have axonal output branches in the flight neuropile of the metathoracic ganglion. Thus it is probable that all these neurones

contribute to the generation of the ESPSs in the hindwing elevator motoneurones. Owing to the difficulty of routinely recording simultaneously from two neurones within a single ganglion, we have been unable to determine the contribution (if any) of each of these interneurones to the EPSP in hindwing elevator motoneurones.

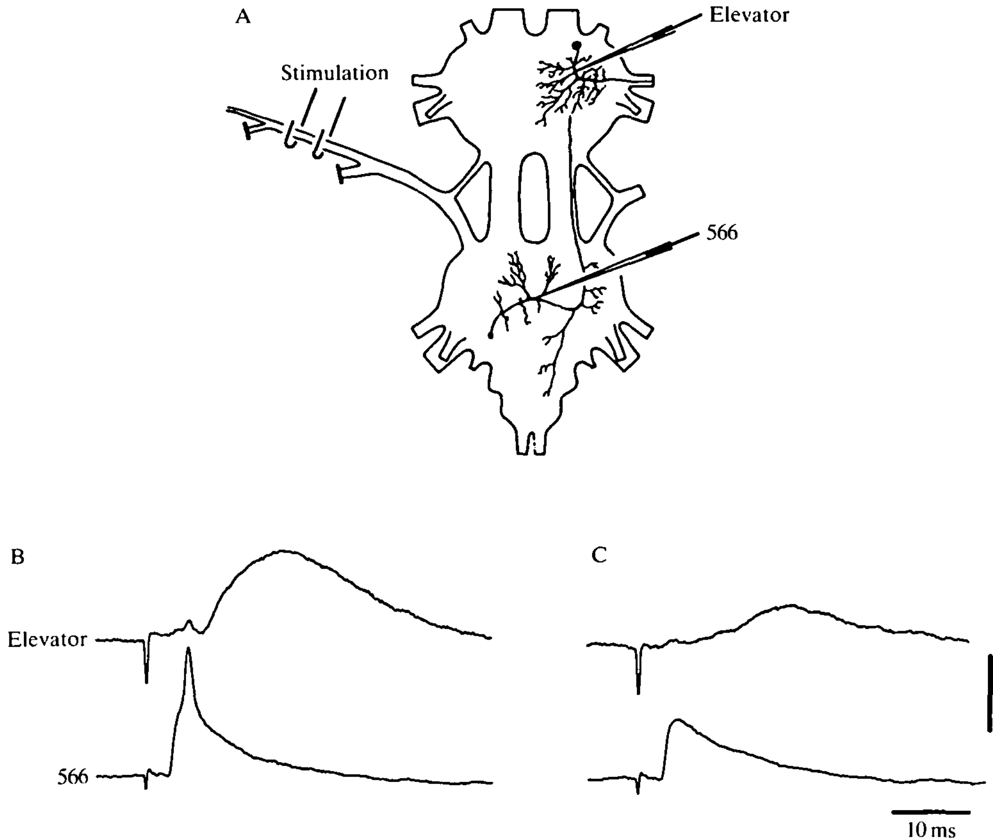

Fig. 10. Demonstration that interneurone 566 is a major element in the excitatory pathway from the hindwing tegulae to forewing elevator motoneurones. (A) Diagram of experimental set-up. Simultaneous recordings were made from interneurone 566 and an elevator motoneurone in the mesothoracic ganglion and tegula afferents were activated by stimulating one metathoracic nerve 1C. (B,C) Potentials recorded intracellularly from the mesothoracic elevator motoneurone (top traces) and interneurone 566 (bottom traces) in response to single stimulus pulses (indicated by initial negative artefact) applied to nerve 1C. (B) With no current injected into interneurone 566 the stimulus strength was adjusted to evoke a single action potential in interneurone 566 (this was below the strength required for activation of wing afferents, see Fig. 2D). (C) A small hyperpolarizing current was injected into interneurone 566 to block the generation of the action potential. Note that blocking the spike in 566 reduced but did not abolish the excitatory response in the elevator motoneurone. The small positive potentials in the recordings from the elevator motoneurone corresponding to the rising phase of the EPSP and the spike in 566 were due to capacitive coupling between the recording electrodes. Vertical scale bar, top traces 4 mV, bottom traces 10 mV.

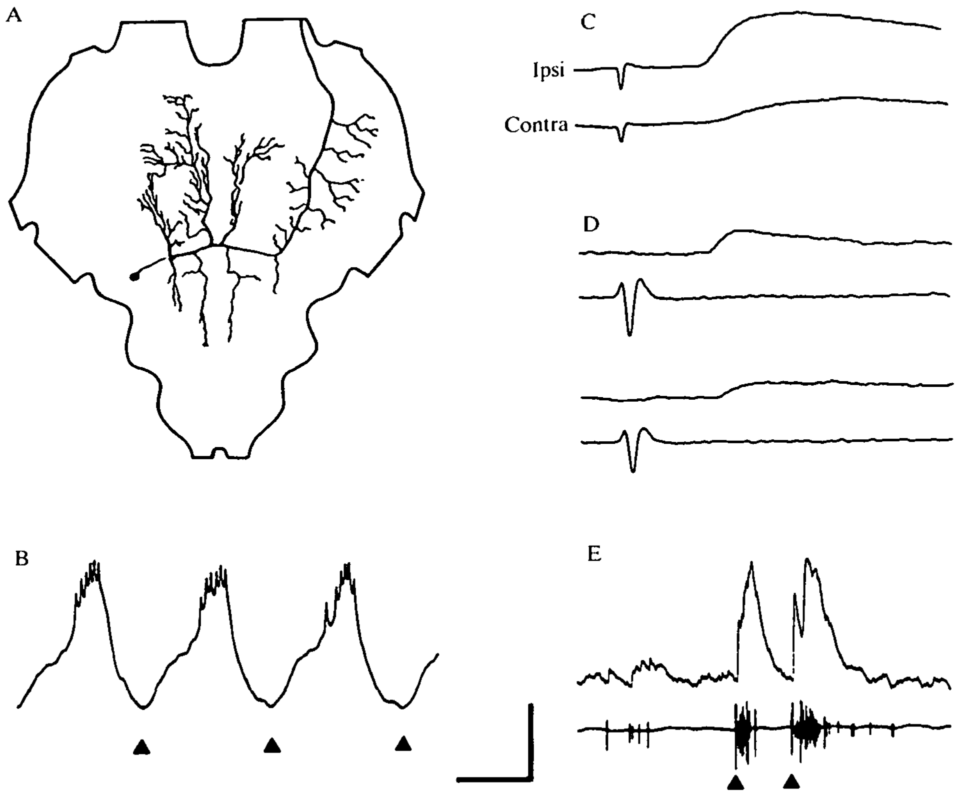

Fig. 11. Excitatory interneurone 504 receives excitatory input from the hindwing tegulae. (A) Drawing of 504 in the metathoracic ganglion. (B) Oscillations in the membrane potential of 504 in response to a wind stimulus directed onto the head in a deafferented preparation. The triangles indicate the time of depressor activity. (C) EPSPs evoked in 504 by electrical stimulation of the tegula nerves (N1C1a) ipsilateral (ipsi) and contralateral (contra) to the soma. (D) Two examples of unitary EPSPs (top traces) evoked in interneurone 504 by spikes in afferents from the ipsilateral tegula (bottom traces are recordings from nerve 1C1a). (E) Depolarization of interneurone 504 (top trace) produced by touching the ipsilateral tegula. Tegula activity was recorded from nerve 1C1a (bottom trace). The triangles indicate the onset of tegula activity in response to touching the tegula. Calibrations: vertical (intracellular recordings) B 20 mV, C 10 mV, D 4 mV, E 5 mV; horizontal B 50 ms, C,D 4 ms, E 125 ms.

However, we did observe on one occasion that interneurone 566 made a relatively weak connection to a hindwing anterior tergocoxal motoneurone. The EPSP amplitude (about 1 mV) was considerably smaller than the EPSPs evoked by spikes in interneurone 566 in forewing elevator motoneurones.

Inhibitory interneurones

In this study we found that an interneurone which in previous studies had been shown to inhibit depressor motoneurones received strong excitatory input from both hindwing tegulae (Fig. 12). This interneurone has been labelled 511 (Robertson &

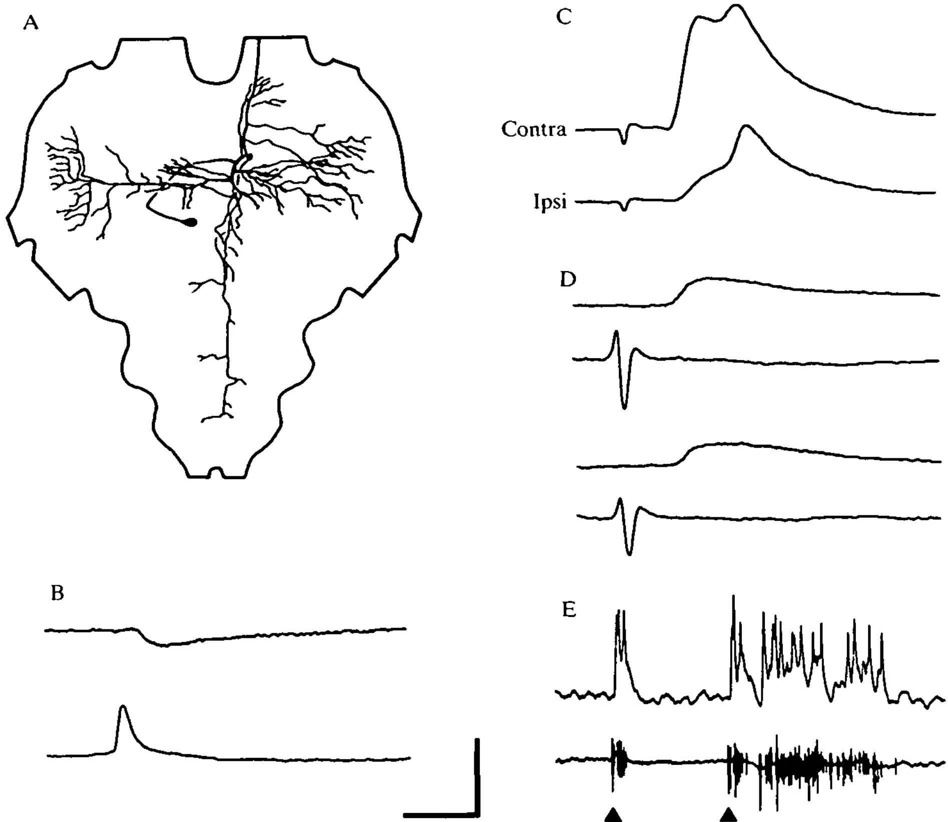

Fig. 12. Tegula afferents excite an interneurone that inhibits depressor motoneurones. (A) Drawing of inhibitory interneurone 511 in the metathoracic ganglion. (B) Inhibitory connection of interneurone 511 to a forewing depressor motoneurone. A spike in 511 (bottom trace) is followed by an IPSP in the forewing first basalar motoneurone. (C) Electrical stimulation of nerve 1C contralateral (contra) and ipsilateral (ipsi) to the soma of 511 evoked EPSPs and spikes in interneurone 511. Two spikes were generated with contralateral stimulation but the first was obscured by the large EPSP. Note the stronger contralateral input corresponding to the more extensive contralateral arborizations. The initial negative deflections in these records are stimulus artefacts. (D) Two examples of unitary EPSPs evoked in 511 (top traces) by spikes in contralateral tegula afferents (bottom traces are recordings from nerve 1C). (E) Touching the contralateral tegula results in a strong excitation of interneurone 511. Top trace, intracellular recording from 511. Bottom trace, extracellular recording from contralateral nerve 1C. The triangles indicate the onset of activity in tegula afferents in response to touching the tegula. Calibrations: vertical (intracellular recordings) B 7·5 mV, C–E 10 mV; horizontal B 8 ms, C,D 4 ms, E 125 ms.

Pearson, 1983, 1985). Large EPSPs, with latencies similar to those evoked in ipsilateral hindwing elevator motoneurones, were evoked in interneurone 511 by stimulating hindwing tegula afferents (Fig. 12C). There was also a 1:1 correspondence of extracellularly recorded spikes from tegula afferents in nerve 1C and unitary

EPSPs in the interneurone (Fig. 12D), and gently touching the tegulae strongly excited the interneurone (Fig. 12E). These observations indicate that this interneurone receives direct connections from hindwing tegula afferents.

Spikes could readily be evoked in interneurone 511 by electrical stimulation of hindwing tegula afferents (Fig. 12C). This observation indicated that interneurone 511 contributed to the generation of the IPSPs in depressor motoneurones. On one occasion we were able to demonstrate this contribution by blocking spike initiation with hyperpolarizing current and noting a reduction in the amplitude of the evoked IPSP in a forewing depressor motoneurone (first basalar).

Another interneurone that may contribute to the production of IPSPs in depressor motoneurones is interneurone 515 (Fig. 13A). The soma of interneurone 515 is located in the ventromedial region of the metathoracic ganglion, and its processes are located in the dorsal neuropile region. The main neurite crosses the ganglion in dorsal commissure V and its axon projects anteriorly in the lateral dorsal tract. Processes arise from the main axon and these project to both sides of the meta- and mesothoracic ganglia. During rhythmic motor activity interneurone 515 discharges a high-frequency burst in-phase with the elevators. Corresponding to this pattern of

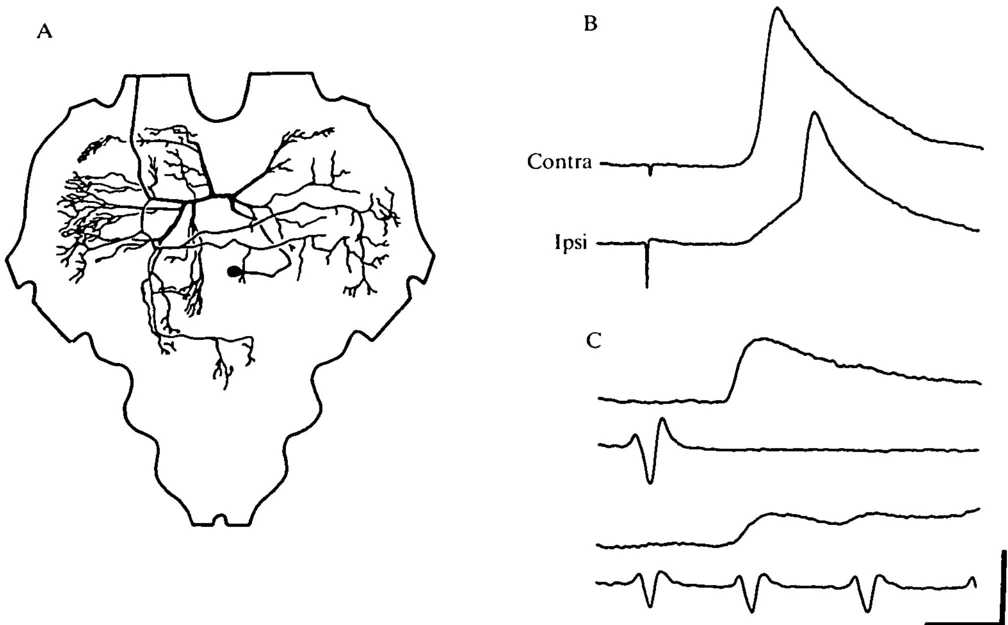

Fig. 13. Inhibitory interneurone 515 receives excitatory input from both hindwing tegulae. (A) Drawing of the interneurone 515 in the metathoracic ganglion. (B) EPSPs and spikes evoked in interneurone 515 by electrical stimulation of the tegula nerves contralateral (contra) and ipsilateral (ipsi) to the soma. (C) Unitary EPSPs (top traces) evoked in interneurone 515 by spikes in single afferents (bottom traces) from the contralateral (top pair) and ipsilateral (bottom pair) tegula, respectively. The oscilloscope was triggered from the afferent spikes. Calibrations: vertical (intracellular recordings) B 10 mV, C 4 mV; horizontal B, C 4 ms.

activity was our observation (on three occasions) that spikes in interneurone 515 produced short-latency IPSPs in forewing depressor motoneurones. We also found that interneurone 515 received strong excitatory input from both hindwing tegulae (Fig. 13B). The connections of tegula afferents to 515 are probably monosynaptic since unitary EPSPs followed 1:1 single spikes in tegula afferents (Fig. 13C).

Inhibitory connections to interneurones

In the course of our investigation on the connections of tegula afferents with interneurones we observed that a number of previously identified interneurones in the flight system received inhibitory input from the hindwing tegulae, namely interneurones 201, 302, 501 and 701 (see Robertson & Pearson, 1983, 1985, for detailed descriptions of these interneurones). All four of these interneurones discharge strongly in-phase with depressor activity. Interneurones 201 and 701 make excitatory connections to depressor motoneurones, while interneurones 302 and 501 make inhibitory connections to elevator motoneurones (Robertson & Pearson, 1983, 1985). Electrical stimulation of either hindwing tegula nerve evoked IPSPs in all four interneurones. The latencies of the IPSPs were about 7 ms, similar to the latencies of IPSPs evoked in depressor motoneurones and signifying that the connections from tegula afferents are not monosynaptic.

Effects of tegula ablation on the flight motor pattern

The strong excitatory connections of tegula afferents to elevator motoneurones and the inhibitory influence on depressor motoneurones, together with the fact that tegula activity commences just prior to elevator activity and is maintained throughout it (unpublished observation; Neumann, 1985), suggest that the input from the hindwing tegulae functions to initiate activity in elevator motoneurones. To test this we recorded the EMG patterns in forewing and hindwing elevator and depressor muscles in animals following surgical removal of the hindwing tegulae and compared these patterns with those produced in intact animals. As expected, the time interval between the onset of depressor activity and the onset of the following elevator activity increased (Fig. 14). In intact animals this interval was about 20 ms and in some animals it was almost independent of wingbeat frequency. Following ablation of the tegulae the depressor to elevator interval increased to about 40 ms and consistently became dependent on the wingbeat frequency. Corresponding to the increase in the depressor to elevator interval was a decrease in the wingbeat frequency (Fig. 14B,C). The increase in the depressor to elevator interval was not simply a consequence of the slowing of the wingbeat frequency since the phase of the onset of elevator activity in the depressor cycle changed from about 0·45 in intact animals to about 0·6 following ablation of the tegulae, i.e. the change in the depressor to elevator interval was greater than the change in the elevator to depressor interval.

Intracellular recordings from elevator motoneurones in intact tethered flying locusts have shown that the initial activation of these motoneurones is due to a discrete component of synaptic input that is absent in deafferented preparations (Wolf & Pearson, 1987). We have recently observed that this rapid early component

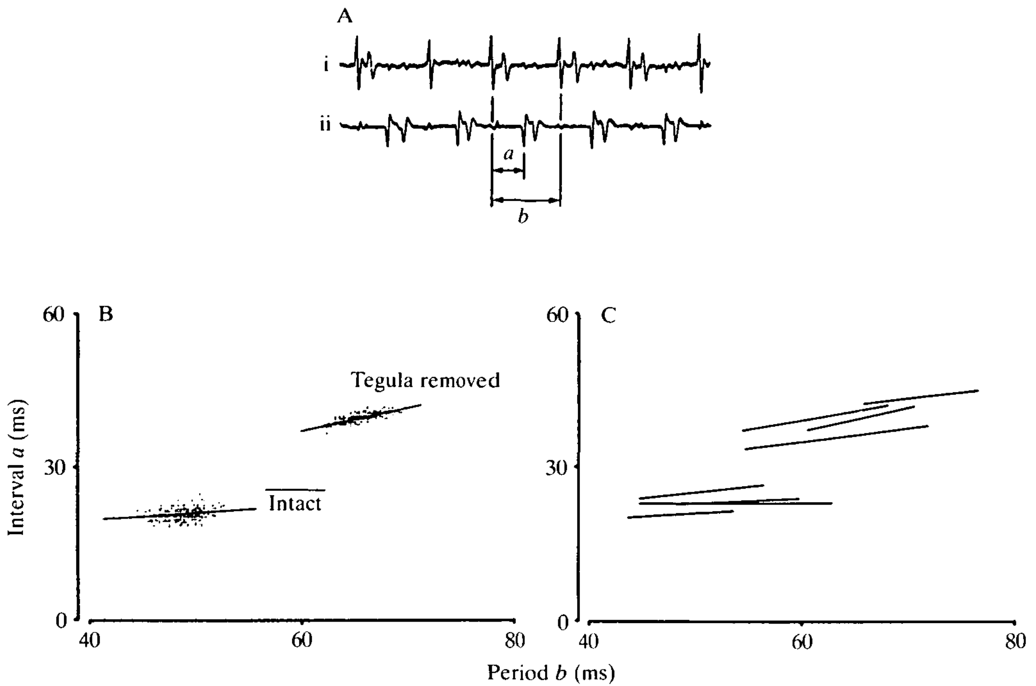

Fig. 14. Effect of hindwing tegula ablation on the timing of activity in hindwing and forewing first basalar (i) and tergosternal (ii) muscles in tethered flying animals. (A) Example of EMG recordings showing the measured parameters: a, interval between the first depressor spike and the first spike in the subsequent elevator burst; b, cycle period. (B) Plots of the depressor to elevator interval showing data points and the best fitting lines for a single trial in an intact animal and in an animal with the hindwing tegulae removed. (C) Plots of best fitting lines for data from single trials in four intact animals (lower set of records) and four animals after hindwing tegula ablation (upper set of lines). Note that the hindwing tegula ablation decreased in wingbeat frequency (i.e. increased the period) and increased the depressor to elevator interval.

is not generated in animals with the hindwing tegulae ablated (H. Wolf & K. G. Pearson, in preparation). The finding that this component is abolished following ablation of the tegulae explains the increase in the depressor to elevator interval, and further supports our conclusion that the tegulae function to initiate activity in elevator motoneurones.

DISCUSSION

In this investigation on the flight system of the locust, *L. migratoria*, the main findings were (i) that there are two distinct groups of afferents arising from the hindwing tegulae, (ii) that tegula afferents make excitatory connections to elevator motoneurones and inhibitory connections to depressor motoneurones, (iii) that some of the interneurones in the excitatory and inhibitory pathways from hindwing tegula afferents to flight motoneurones could be identified, and (iv) that there is a delay in

the onset of elevator activity following ablation of the hindwing tegulae in tethered flying animals. Before considering the functional implications of these findings we will first review our results and consider the possible reasons why the connections we found between tegula afferents and flight motoneurones were not the same as those reported for *S. gregaria* by Kien & Altman (1979).

Two groups of tegula afferents

Electrical stimulation applied directly to the hindwing tegula nerve showed that this nerve contained at least two distinct populations of afferent fibres distinguished by their conduction velocities and thresholds for electrical stimulation (Figs 2, 3). A similar finding has been reported for *S. gregaria* (Kien & Altman, 1979). Based on differences in conduction velocity it can be calculated (see Stein & Pearson, 1971, for the method) that the mean difference in diameters of the two populations is about 3 μm, assuming the largest fibres are 10 μm in diameter (Altman *et al.* 1978). Thus the two groups of afferents we have identified physiologically appear to be subgroups of the afferents with diameters in the range of 5–10 μm (Kien & Altman, 1979). Kutsch *et al.* (1980) reported that each tegula contains two sets of receptors, mechanosensory hairs and a chordotonal organ. It is not known whether these two sets of receptors give rise to two distinct populations of afferents based on fibre size. Judging from the sizes of spikes recorded from the tegula nerve it appears that the mechanosensory hairs give rise to slightly smaller afferents since waxing them appears to have little effect on the activity of the largest afferents in tethered flying animals (Neumann, 1985). Thus it is conceivable that the two populations of afferents we have identified physiologically do arise from the two groups of receptors associated with each tegula: the large afferents arising from the chordotonal organ and the smaller afferents arising from the mechanosensory hairs.

Tegula connections to flight motoneurones

We have found that both groups of afferents from one hindwing tegula make direct excitatory connections onto elevator motoneurones in the ipsilateral half of the metathoracic ganglion. Our conclusion that the connections are monosynaptic is based on latency measurements (see Results), the 1:1 correspondence of unitary EPSPs following spikes in tegula afferents (Fig. 4), and the overlap of the central processes of tegula afferents with the processes of flight motoneurones (Tyrer & Altman, 1974). The evidence that both groups of afferents make excitatory connections is that threshold stimulation for large afferents always evoked EPSPs in elevator motoneurones (Fig. 4) and that additional recruitment of the group of smaller afferents increased the amplitude of the evoked EPSP (Figs 4, 5). It is conceivable that the increase in EPSP amplitude was due to the activation of a residual group of the larger afferents rather than to activation of the smaller afferents. Two observations make this unlikely: (i) examination of the rising phase of the evoked EPSP showed that the onset of the increase in EPSP amplitude when the smaller afferents were recruited was delayed by an amount equal to the difference in conduction times for the large and small afferents (Fig. 5), and (ii) individual spikes

in both small and large afferents (as judged by the relative sizes of the extracellularly recorded spikes from nerve 1) gave rise to unitary EPSPs in elevator motoneurones (Fig. 4).

Hindwing tegula afferents were also found to make excitatory connections to elevator motoneurones in the mesothoracic and contralateral half of the metathoracic ganglia (Fig. 6). These connections must be *via* interneuronal pathways because the central projections of the hindwing tegula afferents are confined to the ipsilateral half of the metathoracic ganglion (Kien & Altman, 1979). Latency measurements indicate that the connection to these elevator motoneurones is *via* a disynaptic pathway. Similarly, inhibitory input to depressor motoneurones from the hindwing tegula afferents appears to be *via* disynaptic pathways since the latencies of the IPSPs in depressor motoneurones were 2–3 ms longer than those of the monosynaptic EPSPs in ipsilateral hindwing elevator motoneurones.

None of our data showed that elevator motoneurones received inhibitory input from either group of tegula afferents, or that depressor motoneurones can be excited by tegula afferents. For example, IPSPs were never observed in elevator motoneurones with threshold activation of the large tegula afferents, and no decrements in the evoked EPSP amplitude occurred in these motoneurones as the strength of tegula nerve stimulation was increased to recruit the group of smaller afferents.

Comparison with tegula connections in S. gregaria

Our findings concerning the connections made by the hindwing tegula afferents to flight motoneurones differ significantly from those reported by Kien & Altman (1979) for *S. gregaria*. They concluded that the hindwing tegula has two functionally distinct pathways to flight motoneurones in the metathoracic ganglion, the larger tegula afferents inhibiting elevator motoneurones and exciting depressor motoneurones, and the smaller tegula afferents exciting elevator motoneurones and inhibiting depressor motoneurones. Thus the major difference is that in our study on *L. migratoria* we found that large tegula afferents excite elevators and inhibit depressors, whereas Kien & Altman (1979) found the opposite in *S. gregaria*. If both sets of observations are accurate then we must conclude that there is a major species difference in the central connections of hindwing tegula afferents. However, there are aspects of the study by Kien & Altman that cause us to question their conclusion that the large afferents in *S. gregaria* produce IPSPs in elevator motoneurones and EPSPs in depressor motoneurones.

The first question is whether their method of stimulation reliably produced selective activation of tegula afferents. Kien & Altman placed a stimulating electrode through the cuticle so the tip lay close to the tegula nerve. Very large voltages were necessary to activate the tegula afferents (about 20 V compared with 0·3 V in the present study). Since neither the tegula nerve nor the stimulating electrode was electrically isolated from the haemolymph the possibility that stimulus spread activated other afferents must be considered. The reported variability in the characteristics of the potentials recorded from nerve 1C in response to stimulation makes this appear likely. Thus it is conceivable that on some occasions Kien &

Altman stimulated large afferents other than tegula afferents and that these other afferents inhibited elevator motoneurones. However, at present no large afferents are known that make the appropriate connections of the appropriate strength. Stretch receptor afferents give only small EPSPs in depressor motoneurones and afferents from the wing are significantly smaller than those from the tegula (Altman *et al.* 1978).

A more likely explanation for the differences between our results and those of Kien & Altman is that some motoneurones were incorrectly identified by them. In their study motoneurones were identified by antidromic activation in response to electrical stimulation of the different flight muscles and by the 1:1 correspondence of spikes in the motoneurones and the flight muscles. It is our experience that, even with careful placement of EMG electrodes in individual flight muscles, activity in adjacent muscles is easily recorded. Furthermore, without careful monitoring of the activity in many motoneurones the selectivity of antidromic activation of individual motoneurones cannot be judged. Thus we question whether the motoneurones in which IPSPs were observed in response to stimulation of the large tegula afferents were elevators and those in which EPSPs were observed were depressors. One indication that motoneurones may have been misidentified in the study by Kien & Altman is that they never observed an EPSP and an IPSP evoked simultaneously in single motoneurones in response to tegula stimulation. This result would not be expected if the large and small tegula afferents produced opposite effects on individual motoneurones, but it is easily explained if some motoneurones were incorrectly identified. Another indication of a misidentification of motoneurones in the study by Kien & Altman comes from an investigation on *S. gregaria* by Burrows (1976). He reported that electrical stimulation of nerve 1C always evoked EPSPs in ipsilateral elevators and IPSPs in ipsilateral depressors. Even at low stimulus strengths, that would have selectively activated large tegula afferents, Burrows did not observe inhibitory connections to elevator motoneurones. These observations are entirely consistent with our own results in *L. migratoria*. We have recently confirmed Burrows' observations in *S. gregaria* (unpublished). We conclude, therefore, that there is no firm basis for believing that there is a species-specific difference in the connections made by hindwing tegula afferents in *S. gregaria* and *L. migratoria*. In particular, it appears very likely in *S. gregaria*, as in *L. migratoria*, that the largest afferents from the hindwing tegulae excite elevator motoneurones and inhibit depressor motoneurones.

Interneurones in tegula pathways

One aim of this investigation was to determine the organization of interneuronal pathways from the hindwing tegulae to flight motoneurones. Although our analysis of interneuronal pathways was not exhaustive we did identify some of the interneurones in these pathways and establish some general features of the organization of these pathways. An excitatory interneurone in the pathway between hindwing tegula afferents and forewing elevator motoneurones is interneurone 566 (Fig. 8). This interneurone was found to receive excitatory connections from

afferents of both hindwing tegulae (Fig. 9) and to make strong excitatory connections to forewing elevator motoneurones (Fig. 8). That interneurone 566 can contribute to the generation of the evoked EPSPs in forewing elevators in response to hindwing tegula stimulation was demonstrated by the marked reduction in EPSP amplitude when interneurone 566 was hyperpolarized in order to block spike initiation (Fig. 10). The observation that the evoked EPSPs in forewing elevator motoneurones were not completely abolished by blocking spikes in interneurone 566 indicates the participation of other interneurones in the excitatory pathway. Two likely candidates are interneurones 504 and 514. Both these interneurones have been shown to make excitatory connections with forewing elevator motoneurones (Robertson & Pearson, 1983) and we have observed that both receive excitatory input from the hindwing tegulae (Fig. 11 and unpublished observations).

An inhibitory interneurone in the pathway between hindwing tegula afferents and forewing depressor motoneurones is interneurone 511 (Fig. 12). This interneurone receives excitatory input from both hindwing tegulae (Fig. 12D) and it makes inhibitory connections to forewing depressor motoneurones (Fig. 12B; Robertson & Pearson, 1983). On one occasion we observed that blocking spike activity in interneurone 511 reduced, but did not abolish, the IPSP evoked in a forewing depressor motoneurone in response to stimulation of hindwing tegula afferents. Thus the inhibitory pathway to forewing depressors probably involves other interneurones in addition to interneurone 511. Interneurone 515 (Fig. 13) has the appropriate properties for being in the inhibitory pathway, namely strong excitatory input from both hindwing tegulae and inhibitory connections to depressor motoneurones.

Currently we know little about the disynaptic pathways from hindwing tegula afferents to hindwing flight motoneurones. Since interneurones 504, 566, 511 and 515 all have axonal output branches in the metathoracic ganglion it is likely that all these neurones form part of disynaptic excitatory and inhibitory pathways to hindwing elevator and depressor motoneurones, respectively. Indeed, on one occasion we observed that spikes in interneurone 566 gave EPSPs in a hindwing elevator motoneurone. However, the amplitude of these EPSPs was small and only sufficient to account for a small part of the evoked EPSP.

From our analysis of the interneuronal pathways from tegula afferents to flight motoneurones we have reached two conclusions. The first is that a number of interneurones act in parallel to mediate disynaptic excitation and inhibition in elevator and depressor motoneurones, respectively. The exact number of interneurones acting in parallel in each pathway has not yet been determined. The second conclusion is that the overall organization of the connections of hindwing tegula afferents to flight interneurones is consistent with our finding that these afferents excite elevator motoneurones and inhibit depressor motoneurones. Table 1 summarizes the connections we found to eight identified interneurones. Those interneurones that excite elevator motoneurones (504 and 566) and inhibit depressor motoneurones (511 and 515) receive direct excitatory connections from the hindwing tegula afferents, whereas interneurones that inhibit elevator motoneurones (302 and 501)

Table 1. *Connections of tegula afferents to identified flight interneurones*

Interneurone	Phase of activity	Connection of motoneurones*	Input from tegula
504	elevator	excite elevators	EPSP
566	elevator	excite elevators	EPSP
511	elevator	inhibit depressors	EPSP
515	elevator	inhibit depressors	EPSP
201	depressor	excite depressors	IPSP
701	depressor	excite depressors	IPSP
302	depressor	inhibit elevators	IPSP
501	depressor	inhibit elevators	IPSP

The interneurones have been divided into two groups depending on their phase of activity.
* Monosynaptic connection made by interneurone to flight motoneurones (see Robertson & Pearson, 1983, 1985).

and excite depressor motoneurones (201 and 701) are inhibited by input from the hindwing tegulae.

Functional considerations

Currently, little is known about the normal function of the tegulae in either the regulation of wing movements or the patterning of motor activity. In *L. migratoria* Kutsch *et al.* (1980) reported (and we have confirmed) that tegula ablation produced a marked decrease in wingbeat frequency, implying that the tegulae play a role in the production of normal flight motor activity. The only specific hypothesis concerning tegula function was proposed by Neumann (1985). Based on the observations of Kien & Altman (1979) he suggested that under normal flight conditions the activity in excitatory and inhibitory pathways is balanced and tegula input has little effect on the motor output. However, under conditions which perturb normal wing movements, activity in the two pathways becomes unbalanced and the difference is used to produce corrective responses in the motor pattern. This interpretation does not account for the decrease in wingbeat frequency following tegula removal (Kutsch *et al.* 1980) and it is inconsistent with the results of the present study; that is, our failure to find evidence for two functionally distinct pathways from hindwing tegulae to flight motoneurones.

Our results have shown that the hindwing tegulae make strong excitatory connections to all elevator motoneurones and inhibitory connections to all depressor motoneurones. By recording from tethered flying animals Neumann (1985) has shown that the tegula afferents are strongly excited during wing depression, with the onset of their activity preceding the onset of elevator activity and with their activity lasting throughout the elevator phase of activity. These two sets of data suggest that afferent input from the hindwing tegulae is involved in generating the initial depolarization of elevator motoneurones during flight. The hypothesis that afferents in nerve 1C are involved in the initiation of elevator activity was originally proposed

by Burrows (1976). Consistent with this proposal was our finding that removal of the hindwing tegulae delayed the onset of elevator activity with respect to the preceding depressor burst (Fig. 14), and abolished the rapid initial depolarizations that normally occur in elevator motoneurones in intact tethered flying animals (H. Wolf & K. G. Pearson, in preparation).

At present we can only speculate on why it is functionally useful for the onset of elevator activity to depend on tegula input. One reason may be the necessity to limit the amplitude of the wing downstroke. We have observed an increased amplitude of the wing downstroke following tegula ablation (unpublished observations) and it is conceivable that this could lead to a decrease in flight performance, e.g. the generation of less lift or thrust. Another reason may be to ensure that the elevators are activated at a more or less constant interval after depressor activity, regardless of wingbeat frequency (Fig. 14). If the depressor to elevator interval was prolonged significantly at low wingbeat frequencies then this might cause the wings to dwell too long in the depressed position. For effective performance it may be mandatory for the wings to return immediately to the elevated position in order to acquire a favourable profile for the production of lift (Zarnack, 1982).

The proposal that the switching from depressor activity to elevator activity depends on a phasic proprioceptive signal is analogous to the situation in the walking systems of crayfish, insects and cats (Sillar, Skorupski, Elson & Bush, 1986; Pearson, 1976; Bässler, 1986; Grillner & Rossignol, 1978). In all these systems it has been demonstrated that the transition from the powerstroke (stance) to the return stroke (swing) depends on a phasic signal generated near the end of the powerstroke. Presumably this prevents the powerstroke from continuing beyond a point where it is no longer effective or even counterproductive. The same principle might apply to the flight system of the locust.

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