

ARE THE HINDWING CHORDOTONAL ORGANS ELEMENTS OF THE LOCUST FLIGHT PATTERN GENERATOR?

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Summary

1. Anatomical and electrophysiological techniques were used to examine the structure, central nervous connections and discharge patterns of afferents arising from the hindwing chordotonal organs in the locust, *Locusta migratoria*.

2. The hindwing chordotonal organ afferents were found to be sensitive to low-frequency sounds (approx. 3 kHz) and to vibrations of the supporting surface. During flight in tethered animals these afferents were strongly activated. This flight-related activity was weakly modulated and the maximum occurred between depressor bursts.

3. Low-frequency sound pulses were used to activate selectively the hindwing chordotonal organ afferents during rhythmic flight activity in deafferented preparations (all motor nerves cut). Phasic stimulation of the chordotonal organs, time-locked to every cycle, had no effect on the frequency of the centrally generated rhythm as recorded from proximal nerve stumps.

4. Staining of single afferents from the hindwing chordotonal organ showed that they bifurcate in the metathoracic ganglion, with one branch terminating in the ring tract and the other branch projecting to the mesothoracic ganglion via the ipsilateral connective. None of the terminal processes of chordotonal afferents was observed to be located in dorsal neuropile regions containing the processes of flight neurones. Consistent with this finding, the chordotonal afferents were not observed to make synaptic connections to flight interneurones or motoneurones.

5. The main conclusion of this investigation is that the hindwing chordotonal organs do not contribute to the patterning of motor activity for flight. The hindwing chordotonal organs probably function as detectors of vibration and perhaps low-frequency sound.

Introduction

Associated with the wings of the locust are at least six groups of sensory receptors: wing-hinge stretch receptors, tegulae, campaniform sensillae, chordo-

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tonal organs, thoracic hair fields and hair bristles on the wings. Considerable effort has been directed towards determining the role of some of these receptors in patterning motor activity for flight. This has led to a number of specific proposals regarding the function of the stretch receptors (Burrows, 1975, 1976; Möhl, 1985*a,b*; Wendler, 1982; Wolf & Pearson, 1988), the tegulae (Neumann, 1985; Pearson & Wolf, 1988; Wolf & Pearson, 1988), the campaniform sensilla (Horsmann & Wendler, 1985; Elson, 1987*a,b*) and the hair bristles on the wings (Weis Fogh, 1956). Currently we have no knowledge about the function(s) of the chordotonal organs or thoracic hair fields.

The chordotonal organs of the hindwings consist of about 21 scolopidia in close association with each wing-hinge stretch receptor (Gettrup, 1962). The only reported property of the afferents arising from the hindwing chordotonal organ is that they are activated by wing depression (Gettrup, 1962). The effect of this activity on the flight motor pattern has not been established. Indeed, there are at present no indications that the chordotonal organs are involved in patterning flight activity, and at least one finding suggests that the chordotonal organs may be without direct effect on the flight system. This is that stimulation of the hindwing stretch receptor and chordotonal organ afferents produces the same effect on the central oscillator as stimulation of the hindwing stretch receptor alone (Reye & Pearson, 1988).

The aim of this investigation was to establish whether the hindwing chordotonal organs can influence the pattern-generating network for flight. Several approaches were used in this analysis. The first was to determine the timing of activity in the chordotonal organ afferents during tethered flight in intact animals; the second was to stimulate selectively the chordotonal organ afferents and examine the effect on the centrally generated rhythm in deafferented preparations; and the third was to determine the central projections and connections of the chordotonal organ afferents to neurones in the meso- and metathoracic ganglia. The results of this study indicate that activity in hindwing chordotonal organ afferents has no influence on the flight system and that these afferents make no connections to flight interneurones.

Materials and methods

Animals

All experiments were performed on adult male and female *Locusta migratoria* obtained from a colony at the University of Alberta.

Preparations and recording set-up

Two different preparations were used in this investigation. The first (the dorsal preparation) was used to examine the conduction properties and central connections of afferents in nerve 1D2 and to investigate the effect of activating the chordotonal organ afferents on the centrally generated flight rhythm. The second (the ventral preparation) was used to determine the activity patterns in th

chordotonal organ afferents in response to sound pulses and to passive and active wing movements.

Dorsal preparation

Following the removal of the wings and legs the animals were mounted dorsal side up on a cork board. The thoracic ganglia were exposed by making a midline incision along the dorsum of the thorax and removing the underlying gut, fat, salivary glands and small muscles. The meso- and metathoracic ganglia were covered with saline and a rigid stainless-steel plate was placed under them.

To determine the conduction properties and connections of afferents in nerve 1D2, monopolar hook electrodes (75 μm silver wire) were placed both distally on nerve 1D2 and proximally on nerve root 1 on one side of the animal. Nerve 1D2 was exposed by removing the hindwing dorsal longitudinal muscle. Both electrodes were covered with silicone grease to insulate them from the saline. The distal electrode was used to stimulate the afferents in nerve 1D2 and the proximal electrode on nerve 1 was used to monitor the afferent input to the ganglion. The distance between the two electrodes was about 6 mm.

The central connections of the afferents in nerve 1D2 were determined by recording intracellularly in neurones in the meso- and metathoracic ganglia and observing the response either to electrical stimulation of N1D2 or to low-frequency sound pulses (see below). Recording electrodes were filled with Lucifer Yellow (5 % in distilled water). This dye was injected into individual neurones following recording (-5 nA for about 5 min). The ganglia were removed from the animal, fixed in 4 % paraformaldehyde, dehydrated, and cleared in methyl salicylate. Filled neurones were viewed as wholemounts under epifluorescent illumination. The structure and central projections of individual afferents in nerve 1D2 were determined by penetrating the afferent close to nerve root 1. Lucifer Yellow was injected and the metathoracic ganglion was fixed and cleared in the described manner. Any ganglion found to contain a well-stained afferent was embedded in Spurr's resin and serially sectioned to determine the location of the processes within the ganglion.

The dorsal preparation was also used to examine the influence of phasic input from the hindwing chordotonal organs on the centrally generated flight rhythm. Rhythmic activity was induced by a constant wind stream on the head (see Robertson & Pearson, 1982). To prevent contractions of the flight muscles from mechanically stimulating the chordotonal organs, the dorsal longitudinal muscles were removed (without cutting nerves 1D2) and all the motor nerves with the exception of metathoracic nerves 1 were cut. Metathoracic nerves 6 and 1C were also transected to eliminate input from the tympanal organs and tegulae, respectively. Extracellular hook electrodes were placed on one metathoracic nerve 1 to monitor flight activity (indicated by large spikes from motor axons supplying the dorsal longitudinal muscles) and input from the chordotonal organs. The dorsal longitudinal nerve spikes were used to trigger low-frequency (4 kHz) sound

pulses. These sound pulses selectively activated afferents arising from the hindwing chordotonal organs (see below).

Ventral preparation

The ventral surface of the thorax was fastened by wax to a rigid support and the animal was mounted ventral side up in a manner that allowed the wings to move naturally (see Wolf & Pearson, 1987, for details). A small piece of cuticle was removed from above the metathoracic ganglion and the nerve roots 1 were exposed to the point where they divide into branches 1C and 1D. Metathoracic nerve 1C on one side of the animal was cut to eliminate input from the tegula and wing on that side. An extracellular hook electrode was placed on nerve 1 on the same side to monitor activity in the afferents in nerve 1D2 during flight. Flight activity was initiated by directing a wind stream towards the head.

Stimulation of afferents in nerve 1D2

The metathoracic nerves 1D2 contain afferents arising from the stretch receptor, chordotonal organ and a number of thoracic hair fields (Altman *et al.* 1978). Electrical stimulation (0.2 ms pulses) of nerve 1D2 was used to activate these afferents in some experiments. The stretch receptor could easily be stimulated selectively (see Fig. 1) because it is significantly larger than all other axons in nerve 1D2 (Altman *et al.* 1978). The afferents arising from the chordotonal organ and the hair fields do not form two distinct populations and thus it was not possible precisely to set the stimulation strength so that only one of these two groups of afferents was stimulated (see Fig. 1A). Nevertheless, the largest afferents from the chordotonal organ are significantly larger than the largest afferents from the hair fields (Gettrup, 1962). By monitoring the evoked activity in nerve root 1 it was therefore possible to adjust the stimulus strength to excite selectively the largest chordotonal organ afferents in addition to exciting the stretch receptor axon. Thus, to distinguish the central effects of the three groups of receptors three basic stimulus conditions were used: (i) activation of the stretch receptor alone, (ii) activation of the stretch receptor plus the low-threshold afferents from the chordotonal organ, and (iii) activation of all afferents by high stimulus strengths.

In the course of this investigation we discovered that the hindwing chordotonal organs are very sensitive to low-frequency sounds (see Fig. 2). The response of the chordotonal organ was tested using a broad-band speaker system (Audax HD13D37) in the range 0.5–11 kHz and using piezoelectric speakers (Motorola type PH10) in the range 12–20 kHz. The sound sensitivity of the organ enabled us selectively to activate the chordotonal organ afferents by delivering sound pulses from two speakers placed on each side of the animal. The results obtained by electrical stimulation of low-threshold chordotonal organ afferents and by the delivery of sound pulses were consistent with each other.

Results

Afferents in nerve 1D2

Anatomical studies have shown that the hindwing nerve 1D2 contains approximately 400 axons (Altman *et al.* 1978). The largest of these (about $6\text{ }\mu\text{m}$ in diameter) arises from the wing-hinge stretch receptor. The chordotonal organ gives rise to about 21 axons with diameters in the range $2.5\text{--}4\text{ }\mu\text{m}$ (Gettrup, 1962; Altman *et al.* 1978). The origin of the smaller axons (diameters less than $2.5\text{ }\mu\text{m}$) has not yet been fully established but many arise from hair fields on the thorax (Altman *et al.* 1978). The distinctly different diameters of the afferents originating from the different receptors was confirmed by recording from the afferents and examining their responses to electrical and natural stimuli. Fig. 1A shows typical potentials recorded extracellularly from nerve 1 in response to electrical stimulation of nerve 1D2. With stimulus strengths of about 0.12 V (0.2 ms duration) the axon of the stretch receptor was selectively activated. The origin of this unitary potential was confirmed by elevating the wing stump and noting that the potentials from the stretch receptor axon were identical to those evoked by just-threshold stimuli to nerve 1D2. The stimulus strength required for activating the stretch receptor axon was taken as the reference, T , for establishing the electrical thresholds for activating other afferents in nerve 1D2. The voltage required to just activate the next largest afferents in nerve 1D2 was about 1.3 times the stretch receptor threshold voltage, i.e. $1.3T$. The conduction time of these afferents over the 6 mm distance separating the electrodes was longer than the conduction time of the stretch receptor axon (4 ms vs 2 ms). This resulted in two distinct components in the evoked potential recorded from nerve 1, the first being from the stretch receptor axon. The amplitude of the longer-latency component progressively increased as the stimulus strength was increased (Fig. 1A) and usually remained triphasic. Thus, the voltage at which the afferents other than those arising from the chordotonal organ were excited could not be distinguished by a noticeable change in the second component. However, intracellular recordings from interneurons not receiving input from the large chordotonal afferents indicated the existence of a group of smaller afferents with a threshold voltage of about $1.6T$ (see Fig. 9). We concluded, therefore, that within the range $1.3T\text{--}1.6T$ electrical stimulation of nerve 1D2 could be used to excite selectively the larger chordotonal organ afferents.

Confirmation that the afferents from the chordotonal organ had a larger diameter than had the hair afferents was achieved by measuring the amplitude of extracellularly recorded spikes from nerve 1 in preparations in which all the branches of nerve 1, except branch 1D2, had been cut (Fig. 1B–D). Mechanical stimulation of single hairs in the thoracic hair fields (see Altman *et al.* 1978 for positions) evoked a train of potentials with amplitudes smaller than most of the spontaneously active units (Fig. 1B). Low-frequency sound increased the discharge rate of the spontaneously active units and recruited additional units that were much larger than the hair afferents (Fig. 1C). Covering all the hair fields with wax did not abolish this recruitment of larger units by sound (Fig. 1D). Since

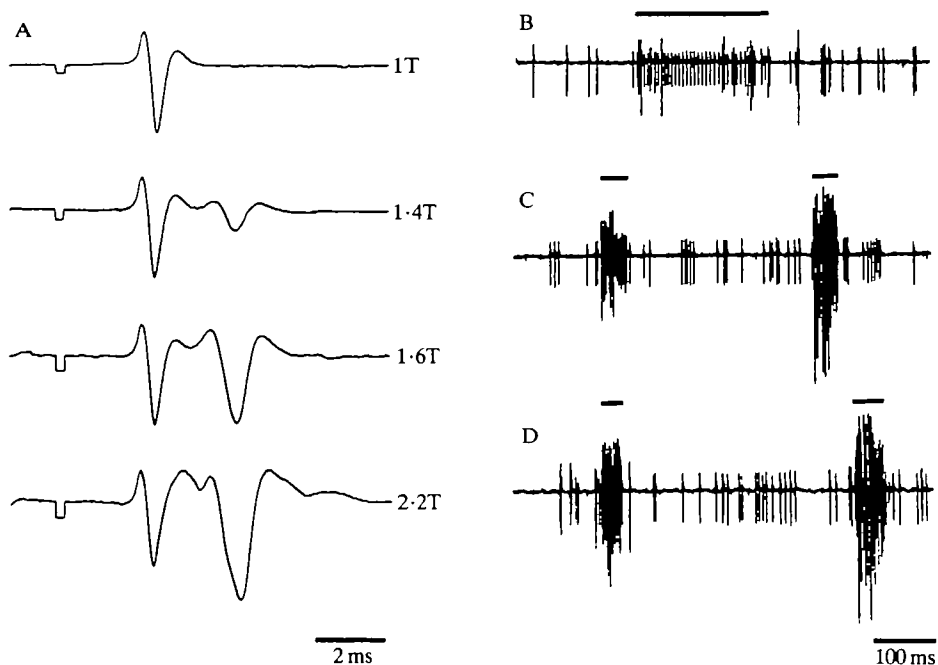


Fig. 1. Physiological characterization of afferents in metathoracic nerve 1D2. (A) Recordings from nerve root 1 show the afferent volley evoked by electrical stimulation of nerve 1D2. The stimulus voltage required to just activate the stretch receptor axon, T, was taken as reference for establishing the voltages required to activate other afferents (top trace). Selective activation of the stretch receptor axon occurred over the range 1.0T–1.3T. Above 1.3T additional, slower-conducting axons were progressively recruited, yielding a second component in the recordings (traces labelled 1.4T, 1.6T and 2.2T). The afferents contributing to this second component arise from the chordotonal organ and thoracic hair fields. (B–D) Responses recorded from nerve 1D2 to (B) deflecting a single thoracic hair; (C) low-frequency sounds with the thoracic hair fields exposed; and (D) low-frequency sounds with the thoracic hair fields waxed over. The responses are indicated by the solid bars above the records. Note that the amplitude of the spikes originating from the hair afferent is smaller than that of the spikes from the spontaneously active units in nerve 1D2 (these units are from the chordotonal organ) and that waxing the hair fields does not abolish the activation of the sound-sensitive units.

waxing the hair fields presumably prevented any activation of the hair sensilla by sound we conclude that the chordotonal organ can be excited by low-frequency sound and that most of the chordotonal organ afferents are larger than the hair afferents. We also noted that the larger afferents were extremely sensitive to vibrations produced by lightly tapping the supporting platform.

Response of chordotonal organ afferents to sound

To investigate the auditory sensitivity of the chordotonal organ afferents, recordings were made from nerve 1 in a ventral preparation with the ipsilateral

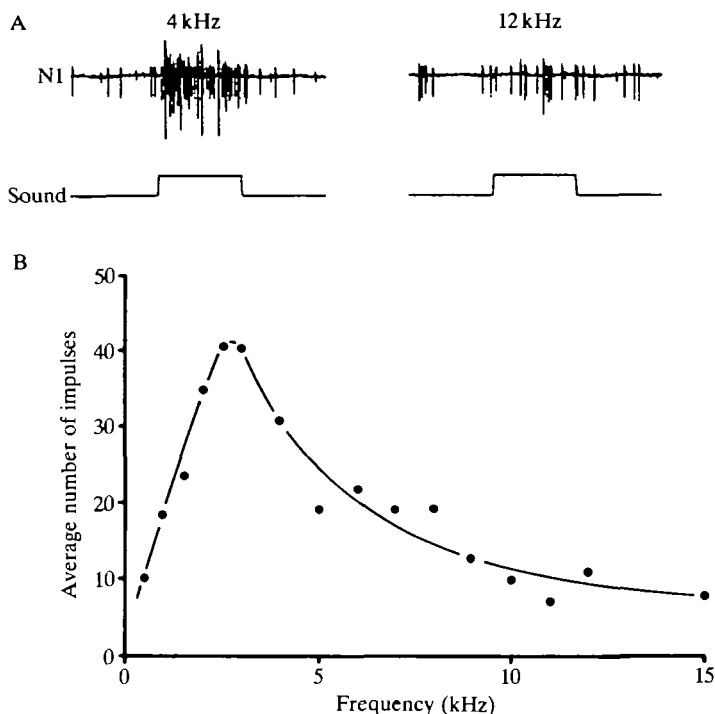


Fig. 2. Sensitivity of afferents in metathoracic nerve 1D2 to sound stimuli. (A) Records from nerve 1 (N1) show that low- (4 kHz) but not high-frequency (12 kHz) sound pulses excite numerous afferents in nerve 1D2 (nerve 1C was cut to eliminate input from the tegula and wing). (B) Frequency-response curve for the sound-sensitive afferents in nerve 1D2. The data for this curve were derived from four animals using sound pulses of 100 ms duration and 70 db re SPL. About 400 stimuli were given at each frequency and the average number of impulses per stimulus was calculated. The activity at frequencies above about 10 kHz is due to spontaneous background activity and not to auditory stimulation.

nerve 1C cut to eliminate input from the wings and tegulae. We assume that removal of the small piece of ventral cuticle in this preparation minimized any change in the mechanical properties of the thorax that might influence the auditory responsiveness of the chordotonal organ afferents. Low-frequency (2–5 kHz) sound pulses evoked vigorous activity in the chordotonal organ afferents (Fig. 2A, left), whereas high-frequency (10–20 kHz) pulses with the same intensity were almost without effect (Fig. 2A, right). The dependence of the chordotonal organ response on frequency is shown in Fig. 2B.

Discharge pattern of chordotonal organ afferents in response to passive and active wing movements

Owing to the high sensitivity of the chordotonal afferents to vibrational stimuli, any passive movement of the hindwing, regardless of direction, resulted in intense activity in these afferents. However, this activity quickly adapted when the wing

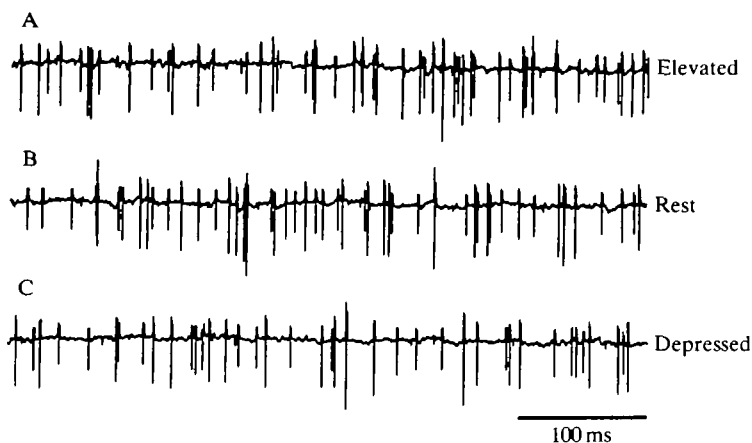


Fig. 3. Maintained elevation or depression of the hindwing does not influence the activity in afferents in metathoracic nerve 1D2. Recordings are from nerve root 1 with nerve 1C cut. (A) Wing held in elevated position. This record was obtained following adaptation of activity in the stretch receptor. (B) Wing folded in normal resting position. (C) Wing held in depressed position.

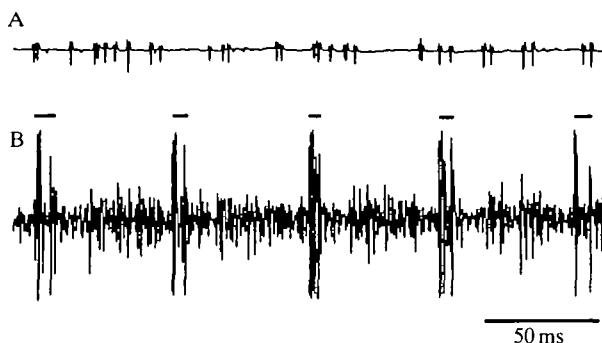


Fig. 4. Activity of afferents in metathoracic nerve 1D2 at rest (A) and during flight in a tethered animal (B). Records are from nerve root 1 with nerve 1C cut. The large spikes in B (indicated by bars) arise from the axons of the dorsal longitudinal motoneurons and the hindwing stretch receptor. Note the intense afferent activity between depressor bursts (compare with A) and the weak modulation of this activity.

was moved and held stationary. An unexpected result was that the spontaneous activity in the chordotonal organ afferents following adaptation was not related to wing position (Fig. 3). In contrast, it has been reported that chordotonal units in *Schistocerca gregaria* are excited during passive wing depression and the adapted level of activity is related to final wing position (Gettrup, 1962).

During flight in tethered animals there was a high level of activity in afferents in nerve 1D2 (Fig. 4B) (for comparison the tonic level of activity in nerve 1D2 in a quiescent locust is shown in Fig. 4A). Although not all the afferent spikes can be

attributed to the chordotonal organ (some may be from hair afferents) there seems little doubt that most of the activity arose from this receptor. This follows from the large amplitude of the spikes (compare Fig. 4A and 4B), similar to those activated by low-frequency sounds. The afferent activity was modulated during flight but in most preparations there was no time when afferent activity was absent. The peak activity always occurred approximately midway between depressor bursts and the minimal level of activity occurred immediately following each depressor burst (Fig. 4B).

Chordotonal organ activity does not influence the flight rhythm

The sound sensitivity of the chordotonal organs allowed us to examine the effects of input from these receptors on the flight rhythm. In a preparation that was *deafferented* (i.e. all motor nerves cut to prevent movement) and had both tympanal nerves cut (metathoracic nerves 6), the hindwing chordotonal organs were stimulated by 40 ms sound pulses triggered at a fixed delay on each cycle by activity in the dorsal longitudinal motor axons (Fig. 5A). Fig. 5B shows that the frequency of the centrally generated rhythm was not influenced when the hindwing chordotonal organs were activated midway through a short flight sequence. The record in Fig. 5B is typical of results obtained in all five animals. This negative result was obtained regardless of the delay between depressor activity and the onset of the sound pulse and regardless of sound intensity and duration.

Our observation that chordotonal organ activity had no effect on the flight rhythm was unexpected. Consequently, we sought additional evidence to demonstrate that the chordotonal organs do not participate in motor pattern generation. This evidence came from an examination of the structure of the central projections of the chordotonal organ afferents and the connections these afferents make with thoracic interneurons.

Anatomy of the central projections of the chordotonal organ afferents

Backfilling the axons of nerve 1D2 with either Lucifer Yellow or cobalt chloride showed that they divide into three major projections in the metathoracic ganglion (Fig. 6A). One projection joins the dorsal medial tract (DMT) anteriorly in the ganglion and runs caudally into the fused abdominal ganglia. This projection shows only minor arborizations off the main tract. A second projection becomes closely associated with the median ventral tract (MVT) and projects to all three abdominal neuromeres. In the metathoracic neuromere, profuse arborizations from this projection engulf the ventral medial tract (VMT) and many additional processes course into the ring tract (RT). Although all other projections are restricted to the ipsilateral half of the ganglion these latter arborizations also supply the contralateral portions of these areas. Minor arborizations occur in the abdominal neuromeres. The third projection enters the meso-metathoracic connective. In the mesothoracic ganglion this branch runs in the MVT and arborizes in the anterior half of the ganglion where it engulfs the VMT and MVT (Fig. 6D–F).

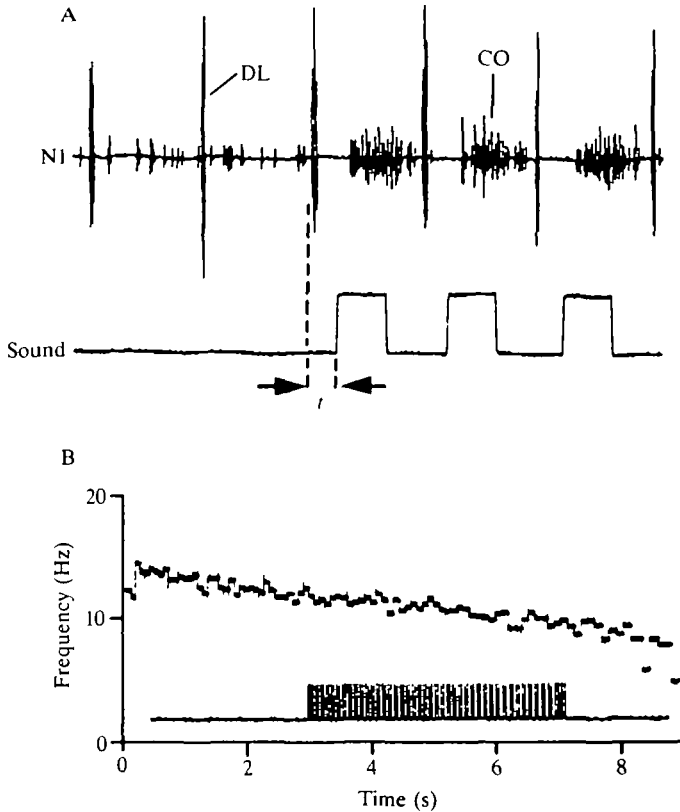


Fig. 5. Phasic stimulation of the hindwing chordotonal organ afferents does not influence the central flight rhythm. Rhythmic flight activity was initiated in a deafferented preparation and low-frequency sound pulses (4 kHz, 95 db, 40 ms) were triggered on each cycle commencing midway through the rhythmic sequence. (A) Records showing that sound pulses (sound) gave an intense burst of activity in the chordotonal organ afferents (CO) as recorded in nerve 1 (N1). The large spikes produced by the dorsal longitudinal motoneurons (DL) were used to trigger the sound pulses at a pre-set delay, t . In this example the delay was 20 ms. (B) Plotting the instantaneous frequency of the central rhythm *versus* time shows that phasic stimulation of the chordotonal organ afferents midway through the sequence did not influence the frequency. The monitor of the sound pulses is shown below the plot. Each step in the plot was calculated by taking the inverse of the cycle period.

To determine which of these projections contained the processes of the chordotonal organ afferents, the structure of single afferents from the hindwing chordotonal organs was determined by penetrating the afferent close to its point of entry into the ganglion and injecting the dye Lucifer Yellow. Penetration of a chordotonal organ afferent was recognized by the 1:1 activation by electrical stimulation of nerve 1D2 and by the occurrence of a brief burst of action potentials in response to a pulse of low-frequency sound (Fig. 7A). The axon of all afferents with these characteristics ($N = 15$) initially projected ventrally in the metathoracic

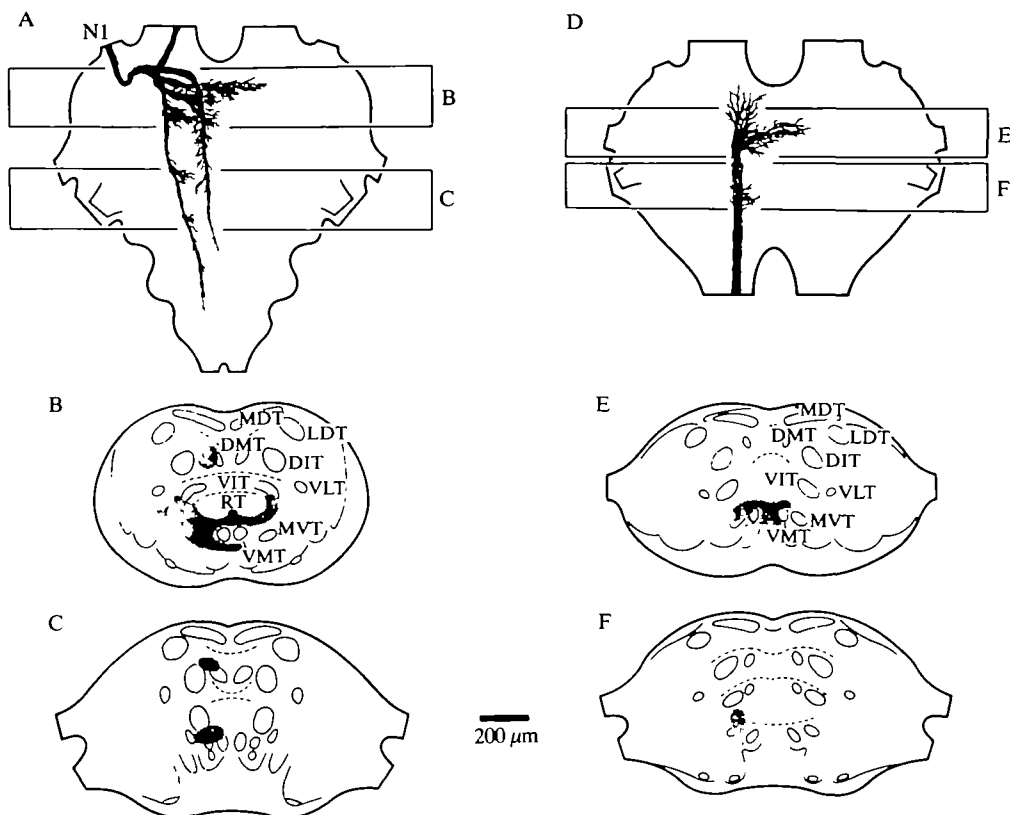


Fig. 6. Central projections of afferents in the metathoracic (A) and the mesothoracic (B) ganglia, as revealed by backfilling axons in nerve 1D2. The locations of the projections relative to the main fibre tracts are drawn into schematic diagrams of the cross-sections of the metathoracic ganglion in B and C, and of the mesothoracic ganglion in E and F. Tracts labelled according to Tyrer & Gregory (1982). MDT, median dorsal tract; LDT, lateral dorsal tract; DMT, dorsal medial tract; VLT, ventral lateral tract; MVT, median ventral tract; VMT, ventral medial tract; RT, ring tract; DIT, dorsal intermedial tract; VIT, ventral intermediate tract.

ganglion, then bifurcated to send a branch into the ipsilateral meso-metathoracic connective. The other branch either entered directly into the frontal auditory neuropile (anterior ring tract) (Figs 7B, 8A) or turned posteriorly before sending secondary processes medially to this neuropile region (Fig. 8B,C). Some of the latter group also projected posteriorly into the fused abdominal ganglia (Fig. 8C). This posterior process was associated with the MVT. We did not attempt to correlate the terminal structure with the auditory characteristics of the afferent.

In the course of determining the structure of single afferents from the chordotonal organ, we also penetrated and stained numerous axons that were not sensitive to sound and were activated only at high stimulus voltages applied to nerve 1D2 ($>1.6T$). None of these axons ($N = 8$) was found to project into the

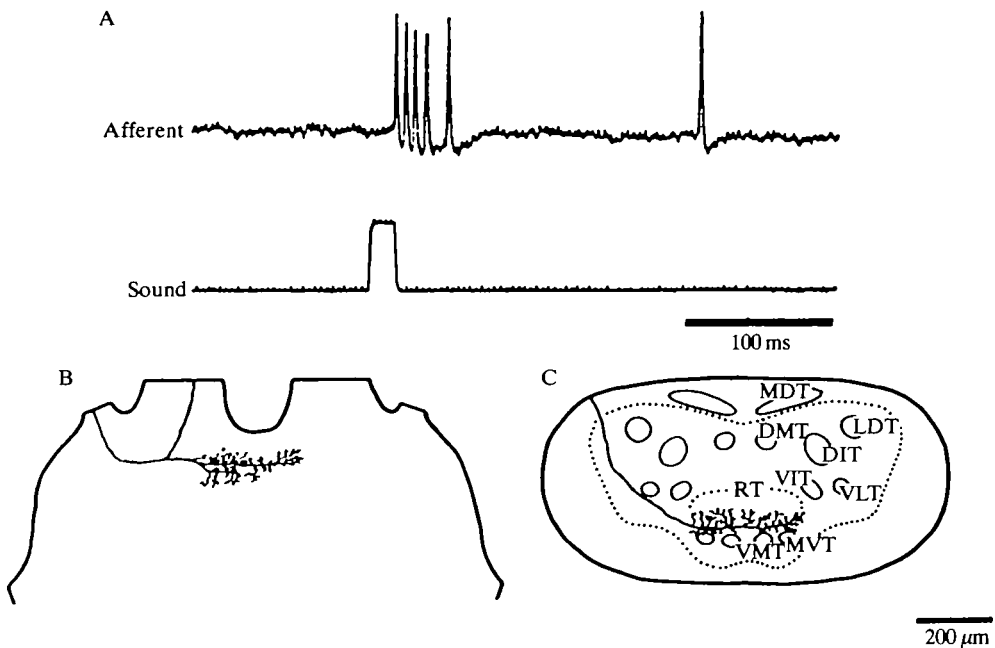


Fig. 7. Recording and staining of a sound-sensitive afferent in the metathoracic nerve 1D2. The afferent was penetrated close to its point of entry into the metathoracic ganglion. (A) A 4 kHz sound pulse evoked a brief burst of action potentials in the afferent. (B,C) Intracellular staining with Lucifer Yellow revealed that the axon bifurcated to send one branch into the meso-metathoracic connective and the other directly into the frontal auditory neuropile (ring tract, RT). Abbreviations as in Fig. 6.

ipsilateral connective or to the frontal auditory neuropile. Instead, all of them initially ran medially then turned to project posteriorly in the DMT. Because of their small size (as judged by their high threshold for activation) and absence of auditory response we presume these afferents arose from the thoracic hair fields.

Central connections of chordotonal organ afferents

If the chordotonal organs are involved in generating flight motor activity then we would expect that they should make connections (either directly or indirectly) onto interneurons in the flight system and/or onto flight motoneurons. To test this expectation the chordotonal organ afferents were excited either electrically, by stimulating nerve 1D2, or acoustically, by low-frequency sound pulses, and intracellular recordings were made from flight motoneurons and interneurons. In none of these recordings did we find evidence for input from the chordotonal organs. A typical example is shown in Fig. 9: interneurone 511 (Fig. 9A) was rhythmically active during flight (Fig. 9B), was not influenced when chordotonal organ afferents were activated by low-frequency sounds (Fig. 9E), and did not receive any input in response to stimulating nerve 1D2 at strengths that activated

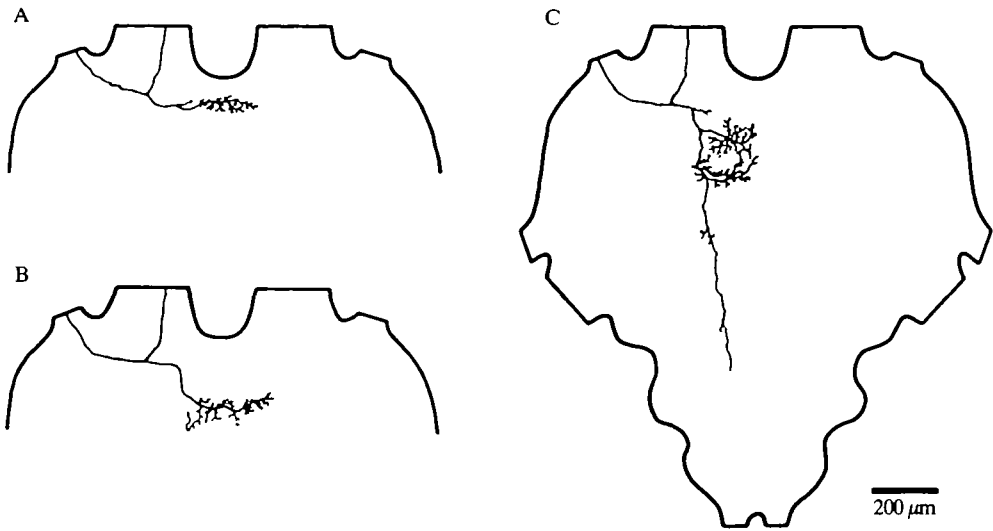


Fig. 8. Variability in structure of the central processes of sound-sensitive afferents in metathoracic nerve 1D2.

the largest afferents (Fig. 9C). Similar results were obtained in all the recordings we made in flight motoneurons and interneurons ($N > 100$).

Although flight neurons were not influenced by activity in the chordotonal organ afferents, many did receive an excitatory synaptic input from smaller afferents (presumably arising from the hair fields). Fig. 9D shows this input for interneuron 511.

The chordotonal organ afferents project to the frontal auditory neuropile (Fig. 7) and so could make connections onto interneurons in the auditory system. One 'auditory' interneuron found to receive strong input from the chordotonal organ afferents was interneuron 601 (Fig. 10A). (This neuron has been labelled as TN1 by Römer & Marquart, 1984.) The characteristic feature of interneuron 601 is that it is strongly excited by low-frequency sounds (Römer & Marquart, 1984). We have found that 601 is also excited by low-frequency sounds following transection of the tympanal nerves (6) and that the depolarizing inputs are temporally related to bursts of activity in the chordotonal organ afferents (Fig. 10B). Electrical stimulation of nerve 1D2 also produced a large EPSP in interneuron 601. This EPSP appeared at stimulus strengths required to activate the largest chordotonal organ afferents (Fig. 10C) and it increased progressively as the stimulus voltage was increased (Fig. 10D,E). The latency of the evoked EPSP following the compound spike recorded from the chordotonal organ afferents in nerve 1 was about 3 ms. Allowing for conduction delay into the ganglion we estimated that the synaptic delay was 1–1.5 ms. Thus, it appears likely that the chordotonal organ afferents make direct, monosynaptic connections with interneuron 601. The overlap of the central projections of the chordotonal organ afferents and the anterior processes of 601 is consistent with this conclusion.

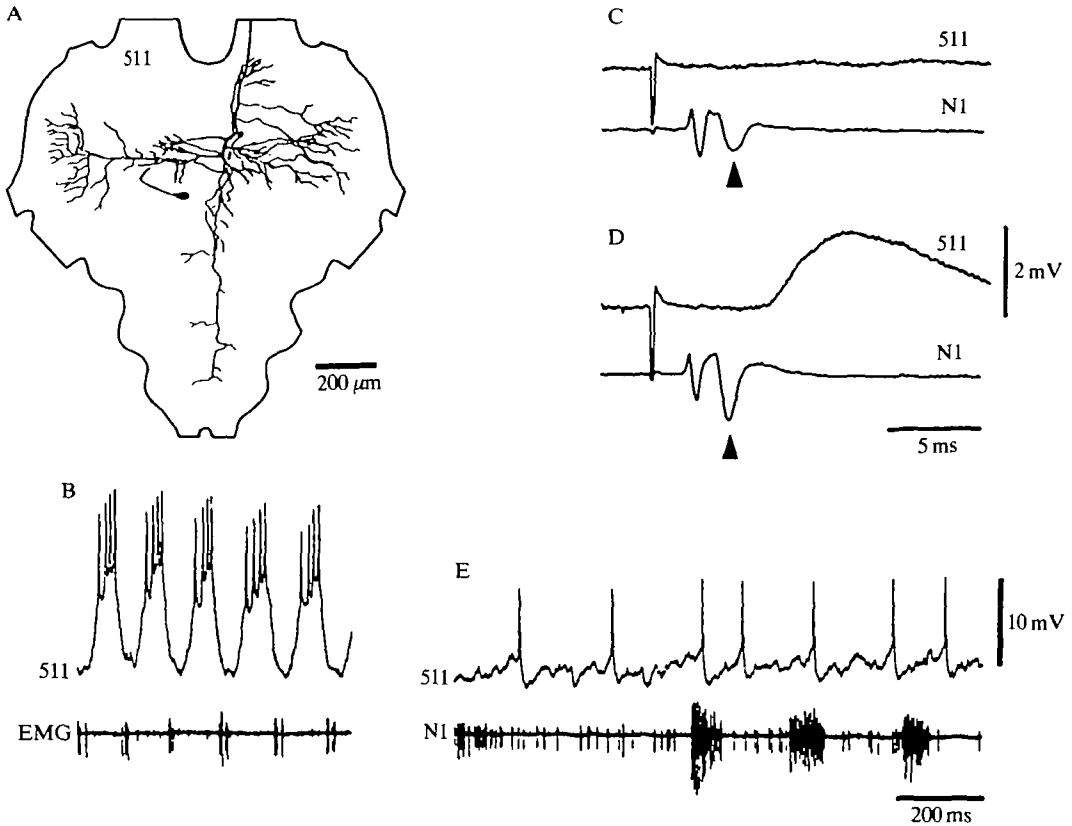


Fig. 9. Flight interneurone 511 does not receive input from the hindwing chordotonal organs. (A) Drawing of interneurone 511 in the metathoracic ganglion. (B) Rhythmic activity recorded intracellularly from interneurone 511 (top trace). The depolarizations occur in antiphase to activity in depressors [bottom trace, electromyogram (EMG) from dorsal longitudinal muscles]. (C) Intracellular recording from neurone 511 (top trace) does not show any signs of synaptic input when nerve 1D2 was stimulated to activate the large afferents of the chordotonal organ. The bottom trace monitors the afferent input *via* nerve 1. The first potential was produced by the stretch receptor axon and the second potential (marked by the arrowhead) represents the afferent volley in the large chordotonal organ afferents. (D) Increasing the stimulus strength to activate all afferents in nerve 1D2 results in an EPSP in interneurone 511. (E) Low-frequency sound pulses (indicated in the bottom trace by intense bursts of activity recorded from nerve 1) do not produce a noticeable depolarization in interneurone 511 (top trace).

In addition to interneurone 601 we found two other interneurons that were strongly excited by the hindwing chordotonal organs. Neither of these interneurons has been described previously. We have labelled these neurones 607 and 608. Drawings of their structure are shown in Fig. 11A,B. Although the two interneurons are in different ganglia they have a number of structural features in common. The cell body of each is located close to the dorsal surface in the anterolateral region of each ganglion, both have axons projecting in the anterior

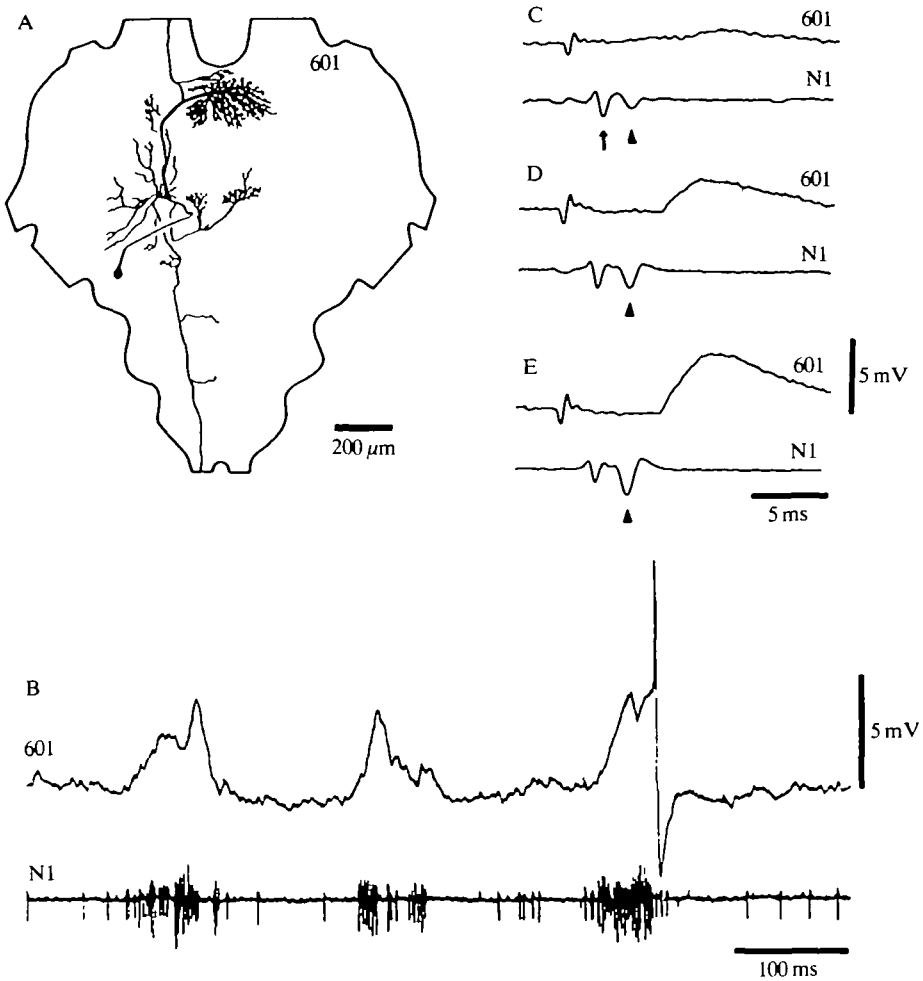


Fig. 10. Excitatory synaptic input from hindwing chordotonal organ afferents to 'auditory' interneurons 601. (A) Drawing of interneurone 601 in the metathoracic ganglion. (B) Depolarization of interneurone 601 in response to low-frequency sound (tympanal nerves cut). Top trace, intracellular recording from 601; bottom trace, extracellular recording from nerve 1. The auditory signals evoked bursts of activity in afferents in nerve 1 and a corresponding depolarization in 601. (C-E) EPSPs evoked in 601 by electrical stimulation of nerve 1D2. Top traces, intracellular recordings; bottom traces, extracellular recordings from nerve 1. In C the stimulus strength was adjusted to excite both the stretch receptor axon (arrow) and the largest afferents of the chordotonal organ (arrowhead). Note the small EPSP evoked by the just-threshold stimulation of the large afferents of the chordotonal organ. As the stimulus strength was increased (indicated by the increase in amplitude of the afferent volley, arrowheads) the amplitude of the EPSP increased (D,E).

and posterior connectives ipsilateral to the cell body, and both have projections into the frontal auditory neuropile regions of the ganglion containing the cell body. The physiological properties of these two interneurons were also similar. Neither

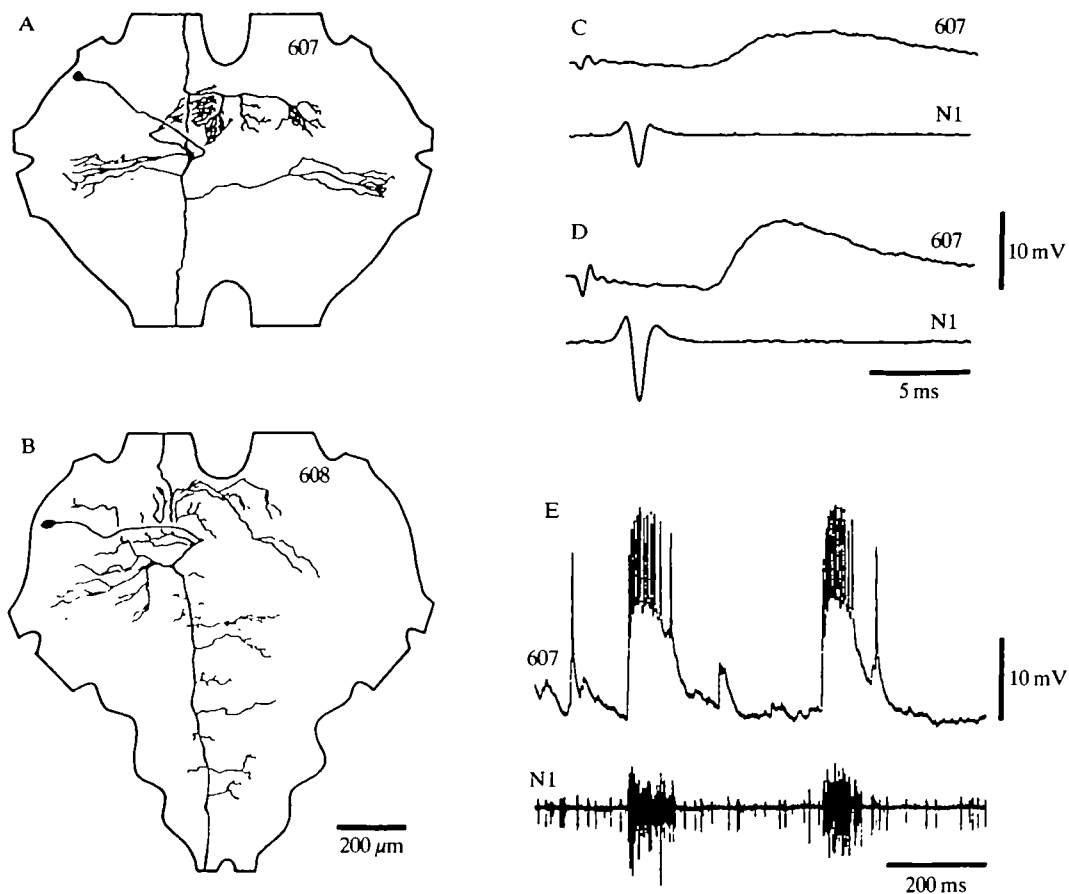


Fig. 11. Interneurone 607 in the mesothoracic ganglion (A) and interneurone 608 in the metathoracic ganglion (B) receive excitatory synaptic input from the hindwing chordotonal organs. (C–D) EPSPs evoked in 607 by electrical stimulation of nerve 1D2. Top traces, intracellular recordings; bottom traces, extracellular recordings from nerve root 1. In this preparation no potential was recorded from the hindwing stretch receptor and thus only a single evoked potential is apparent in nerve 1. Note that as the stimulus strength was increased from C to D (indicated by the increase in amplitude of the afferent volley) the amplitude of the EPSP increased. (E) Strong depolarizations of interneurone 607 in response to low-frequency sounds (tympanal nerves cut). Top trace, intracellular recording from 607; bottom trace, extracellular recording from nerve 1. The auditory signals evoked bursts of activity in nerve 1 afferents and corresponding depolarizations in 607.

was rhythmically active during flight, both were strongly depolarized by low-frequency sounds (with the tympanal nerves cut) and both received a large EPSP in response to electrical stimulation of the hindwing nerve 1D2. Examples of the physiological responses in interneurone 607 are shown in Fig. 11C–E. The similarity of the anatomical and physiological properties of interneurons 607 and 608 suggest that they may be homologous.

The chordotonal organ afferents were also found to make weaker, more variable and longer-latency excitatory connections with a number of other interneurons. Most of these were auditory interneurons that have been described previously. We did not examine the characteristics of these connections in detail.

Discussion

Do the hindwing chordotonal organs contribute to the patterning of flight motor activity?

The main finding of this investigation was that phasic activation of the afferents from the hindwing chordotonal organs had no influence on the flight rhythm produced in deafferented locusts (Fig. 5). Two other findings consistent with this result were: (1) that the chordotonal organ afferents did not terminate in neuropile regions known to contain processes of neurons involved in patterning flight activity, and (2) that the chordotonal organ afferents did not make connections with any interneurons or motoneurons in the flight system. These findings suggest that the hindwing chordotonal organs are not elements in the pattern-generating system for flight. However, this conclusion depends critically on two important assumptions. The first is that afferents from the chordotonal organ all respond to sound and none project to the dorsal neuropile, and the second is that examining the effect of activity in the chordotonal organ afferents on the centrally generated flight rhythm is an appropriate method for assessing the contribution of the chordotonal organs in patterning flight activity. We consider each of these assumptions separately in the following discussion.

Sound-sensitivity of the chordotonal organ afferents

Each hindwing chordotonal organ gives rise to about 21 afferent fibres (Gettrup, 1962). Anatomical studies have shown that these afferents are fairly uniform in diameter ($2.5\text{--}4\text{ }\mu\text{m}$) and that most of them are larger than all other afferents in nerve 1D2 with the exception of the single axon from the stretch receptor (Gettrup, 1962; Altman *et al.* 1978). The only other afferents known to exist in nerve 1D2 arise from thoracic hair fields. Since the spikes from sound-sensitive afferents in nerve 1D2 are larger than the spikes from the hair afferents (Fig. 1) and the chordotonal organ afferents are larger than hair afferents, it follows that the sound-sensitive afferents arise from the chordotonal organ. Supporting this conclusion is the finding that waxing the thoracic hair fields does not prevent sound from activating the larger afferents in nerve 1D2 (Fig. 1E).

Given that the chordotonal organ does give rise to afferents that are sensitive to low-frequency sounds then the next issue is whether all chordotonal organ afferents are sound-sensitive. Unfortunately the total number of afferents in nerve 1D2 that can be excited by sound is impossible to determine. Even sound pulses of modest intensity excite numerous afferents and the summation of spikes from these afferents prevents an accurate determination of the number of afferents

excited (Fig. 1). Nevertheless, examination of extracellular recordings from nerve 1D2 reveals that the minimal number of afferents that can be excited by sound is greater than 10 (K. G. Pearson, B. Hedwig & H. Wolf, unpublished observations). Assuming that only the chordotonal organ afferents are sound-sensitive, then it appears safe to conclude that the majority, but not necessarily all, of the chordotonal organ afferents can be excited by sound. If a sound-insensitive group exists it is unlikely to include the largest afferents from the chordotonal organ. This follows from the failure to find any neurone that could be depolarized by just-threshold electrical stimulation of the large chordotonal organ afferents in nerve 1D2 and not be depolarized by sound stimuli. However, electrical stimulation of nerve 1D2 at high voltages did produce excitatory input to some neurones that were not depolarized by low-frequency sounds (e.g. interneurone 511, Fig. 9). Thus the possibility remains that some of the smaller afferents from the chordotonal organ are not sensitive to sound and that these could influence the flight neurones.

Our anatomical studies have shown that the sound-sensitive and the sound-insensitive afferents in nerve 1D2 have distinctly different structures. All sound-sensitive afferents send projections into the frontal auditory neuropile (ring tract) of the metathoracic ganglion and an axonal branch to the mesothoracic ganglion. By contrast, sound-insensitive afferents are all smaller in diameter (as judged by the larger stimulus voltage required to activate them) and none have central projections to the frontal auditory neuropile region of the metathoracic ganglion (K. G. Pearson, B. Hedwig & H. Wolf, unpublished observations). Assuming that all chordotonal organ afferents are sound-sensitive, then it follows that none of these afferents send projections into the dorsal neuropile regions that contain the processes of neurones in the flight system (Ramirez & Pearson, 1988).

Sensitivity of the method

The issue to be considered in this section is whether our failure to find any effect of the chordotonal organ input on flight activity could be due to the method we used (see Fig. 5) rather than to the chordotonal organs not being involved in pattern generation. In the preparation we employed, all phasic input was eliminated from wing receptors and the frequency of the rhythm was low. It is conceivable that for chordotonal input to have an effect on the flight rhythm it is necessary to have concomitant input from other receptors and/or the oscillator functioning close to normal frequencies. Unfortunately, we have not been able to devise experiments to test these possibilities adequately. However, one argument in favour of the adequacy of the method is that a similar procedure has clearly demonstrated the involvement of the stretch receptors of the forewing and hindwing in pattern generation (Reye & Pearson, 1988). Furthermore, brief stimulation of either the stretch receptors or the tegulae can reset the central rhythm (Neumann, 1985; Reye & Pearson, 1988; Wolf & Pearson, 1988), whereas no resetting effect was observed by presenting low-frequency sound pulses in deafferented preparations with the auditory nerves (6) cut. Corresponding to the

clear effect that the tegulae and stretch receptors can have on the central rhythm is the finding that these receptors send afferent projections into the flight neuropile. By contrast, the chordotonal organ afferents do not influence the central rhythm and do not project to the flight neuropile.

Although we have been unable to observe an influence of chordotonal input on the frequency of the deafferented rhythm, we have not excluded the possibility that chordotonal input could influence more subtle aspects of the motor pattern, such as the relative timing of activity in different motoneurons, without affecting the frequency. To test this possibility will require a detailed analysis of the flight motor pattern with and without chordotonal input.

Conclusion

Although we cannot provide a definite answer to the question posed in the heading of this section, there is considerable evidence that the chordotonal organs are not involved in the generation of the flight rhythm. The main evidence is that phasic input from the chordotonal organs does not influence the centrally generated rhythm, and that chordotonal afferents do not project to the flight neuropile or to flight interneurons or motoneurons. The main uncertainty that prevents full acceptance of this negative conclusion is the possibility that some of the smaller afferents from the chordotonal organ may not be sensitive to sound. If this subgroup of afferents exists then our technique of using sound pulses to activate chordotonal organ afferents would not have activated all the afferents. If this is the case then it is conceivable that the sound-insensitive subgroup could influence the flight system.

Possible function of the hindwing chordotonal organ

The striking feature of the afferents arising from the hindwing chordotonal organs is their sensitivity to low-frequency sound and to vibrations of the supporting platform. The latter property is consistent with the vibrational sensitivity of the chordotonal organ in the forewing base of crickets (Möss, 1971). It suggests that the wing chordotonal organs may function primarily as detectors of vibratory stimuli. This idea is further supported by the connections of the chordotonal organ to the thoracic low-frequency neurone 601. In locusts (Cockl *et al.* 1977), bushcrickets (Kühne, 1982) and crickets (Kühne *et al.* 1984) neurones of the auditory pathway receive additional vibratory input at least from the subgenual organs, which are vibration receptors in the legs. Thus, the hindwing chordotonal organ may be an additional sensory organ feeding information about vibration into the auditory centres of the nervous system. The simultaneous processing of auditory and vibratory stimuli enhances the responses of central auditory/vibratory neurones and is probably an important factor assisting orientation behaviour (Kalmring, 1983).

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