The Journal of Experimental Biology 202, 1579–1587 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
JEB2056

AUDITORY SENSORY CELLS IN HAWKMOTHS: IDENTIFICATION, PHYSIOLOGY AND STRUCTURE

MARTIN C. GÖPFERT* AND LUTZ T. WASSERTHAL

Institut für Zoologie I, Universität Erlangen-Nürnberg, Staudtstraße 5, D-91058 Erlangen, Germany

*Present address: Zoologisches Institut, Universität Zürich, Winterthurerstraße 190, CH-8057 Zürich, Switzerland
(e-mail: mgoepfer@zool.unizh.ch)

Accepted 29 March; published on WWW 20 May 1999

Summary

The labral pilifers are thought to contain auditory sensory cells in hawkmoths of two distantly related subtribes, the Choerocampina and the Acherontiina. We identified and analysed these cells using neurophysiological and neuroanatomical techniques. In the death's head hawkmoth *Acherontia atropos*, we found that the labral nerve carries the auditory afferent responses of a single auditory unit. This unit responds to ultrasonic stimulation with minimum thresholds of 49–57 dB SPL around 25 kHz. Ablation experiments and analyses of the neuronal activity in different regions of the pilifer revealed that the auditory afferent response originates in the basal pilifer region. The sensory organ was identified as a chordotonal organ that attaches to the base of the pilifer. This organ is the only sensory structure in the basal pilifer region and consists of a single mononematic scolopidium and a single sensory cell. In Choerocampina, a homologous scolopidium was also found and is probably the only sensory structure of the pilifer that might serve an auditory function. Since a pilifer chordotonal organ with only a single scolopidium has also been detected in a non-hearing hawkmoth species, hearing in the distantly related choerocampine and acherontiine hawkmoths presumably evolved from a single proprioceptive mechanoreceptor cell that is present in all hawkmoths.

Key words: chordotonal organ, hearing, evolution, insect bioacoustics, ultrasonic hearing, Sphingidae, death's head hawkmoth, *Acherontia atropos*.

Introduction

Within the hawkmoths (Sphingidae), a family of mostly nocturnal Lepidoptera, ultrasonic hearing has evolved at least twice independently in two distantly related subtribes: once in the Choerocampina (Roeder et al., 1968; Roeder and Treat, 1970) and once in some species of the Acherontiina (Göpfert and Wasserthal, 1999). Despite this independent evolution, the ears in species of both taxa share numerous similarities: (1) they are located on either side of the head; (2) each ear is formed by the same two mouthparts, a labial palp and a labral pilifer; (3) the labial palp serves as an accessory auditory structure increasing the acoustic sensitivity; and (4) the auditory sensory cells are thought to be located in the labral pilifers, a pair of small labral projections located lateral to the tongue (Roeder et al., 1970; Roeder, 1972; Göpfert and Wasserthal, 1999).

Given these apparent structural and functional similarities, sound perception in choerocampine and acherontiine hawkmoths probably involves not only homologous head appendages but also homologous sensory structures. The auditory sensory cells of hawkmoths, however, have been neither identified nor characterised. The only studies that focused on these cells were performed by Roeder and Treat (1970) and Roeder (1972), who attempted to record auditory afferent responses in choerocampine hawkmoths. These studies indicated the presence of a single auditory sensory cell in the pilifer of Choerocampina, and the results were later summarised by Roeder (1976) as follows. 'Since the exiting moment when I 'discovered' the pilifer lobe, Asher Treat and I have searched for the acoustic sense cell that it presumably contains and also for the nerve fiber that must conduct impulses from this point to the brain where it appears to connect with the descending pathways. These tasks have been technically difficult, but after many attempts we were able once or twice to register nerve impulses that seemed to come from a single sense cell in the pilifer lobe. But these bits and pieces must await still others to be discovered before they fit together'.

In the present study, we identify and characterise the auditory sensory cells of hawkmoths using neurophysiological and neuroanatomical techniques. Most of the studies were performed in the death's head hawkmoth *Acherontia atropos*, a hearing acherontiine hawkmoth species. In this large species, we analysed auditory afferent responses and localised the sensory structures. To identify the auditory sensory cells in hearing Acherontiina and Choerocampina and to trace the evolutionary origin of these cells, we analysed and compared the presence and distribution of sensory structures in the

pilifers of *Acherontia atropos* and of a hearing choerocampine and a non-hearing acherontiine species.

Materials and methods

Animals and dissections

Three hawkmoths species were studied: the hearing acherontiine species *Acherontia atropos* L., the non-hearing acherontiine species *Panogena lingens* Butler, and the hearing choerocampine species *Hippotion celerio* L. The animals were raised in the laboratory from stocks originating from Kenya (*Acherontia atropos*), Madagascar (*Panogena lingens*) and the Canary Islands (*Hippotion celerio*).

For neurophysiological studies, the animals were briefly anaesthetised with CO_2 prior to removal of the wings and legs, and were waxed dorsum side down to a holder. The head was fixed by waxing the compound eyes to the thorax, and the cervical connectives were transected to minimise movements of the animals.

Two different surgical approaches were used to expose the head nerves for extracellular recordings. During these preparations, acoustic sensitivity was monitored by measuring the acoustically elicited activity of the galeal elevator muscles, as described by Göpfert and Wasserthal (1999). In one of the two surgical approaches used, the head nerves were accessed from the dorsal side. The cuticle in the frontoclypeal region of the head, the pharynx dilator muscles, the frontal ganglion and the pharynx itself were removed, exposing the proximal regions of the suboesophageal and tritocerebral nerves (for general anatomical details, see Eaton, 1988). During surgery, both labial palps and the proboscis remained in their natural position. In the other surgical approach, the head nerves were accessed from the ventral side. Since this side of the head is totally concealed by the labial palps and the proboscis, one labial palp and the distal half of the proboscis had to be removed prior to the dissection. The other palp, however, remained intact, and any changes from its natural position were carefully prevented. The suboesophageal ganglion and the suboesophageal nerves were exposed by removing a flap of labial cuticle between the articulations of both labial palps. Subsequently, the tentorial bridge was crushed lateral to the anterior edge of the suboesophageal ganglion on the side of the head on which the palp remained intact. The tentorial bridge was removed together with the galeal retractor muscle, exposing the tritocerebral nerves on that side of the head. During all dissections, the tissue was continuously submerged in haemolymph saline (Kaissling and Thorson, 1980).

Neuroanatomy

The general anatomy of the nervous system of the head was studied in either preserved (70% ethanol) or freshly killed animals. The identification of nerves was facilitated by staining with Janus Green B (Yack, 1993). To investigate the distal branching of the labral nerve, it was retrogradely filled with cobalt chloride (Altman and Tyrer, 1980). The animal was briefly anaesthetised using CO_2, and the head was removed and transferred to saline. The nerve was exposed from the ventral side, as described above, and cut in its proximal region close to the tritocerebrum. The distal cut end of the nerve was surrounded by Vaseline and filled retrogradely with 1.5 mol l^{-1} $CoCl_2$. After approximately 48 h at 10 °C, the Vaseline was removed and the dye was precipitated with 1% ammonium sulphide. To trace fine projections of the labral nerve inside the pilifer, the nerve was labelled with 0.2 mol l^{-1} $NiCl_2$ in the same manner as in cobalt fills, and the dye was precipitated with rubeanic acid (Quirke and Brace, 1979). For examination and documentation, the pilifer was removed, dehydrated and transferred into benzylbenzoate to clear the cuticle. The presence and distribution of sensory cells in the pilifer were analysed in these whole mounts using a Zeiss Axiovert 35M microscope.

Neurophysiology

All experiments were performed in a 1 m×1 m×1 m Faraday cage lined with foam to minimise acoustic reflections. The animal was positioned in the centre of the chamber, and the speaker was placed in front of the animal at a distance of 0.4 m. The temperature was 20 °C in all experiments.

Acoustic stimuli were pure tones generated by a Voltcraft function generator (model FG 506). The output signal was passed through a digitally controlled attenuator, and the stimulus intensity and duration were controlled by a computer. After power amplification, the signal was fed to a Technics 10TH400C leaf tweeter. The signal was calibrated with the holder in position using a Bruel & Kjaer 4135 microphone (grid off) and a Bruel & Kjaer 2331 sound level meter. Sound pressure levels (SPL) were determined using the 'peak-hold' function of the sound level meter and are given as dB peak SPL (re 20 μPa), which for a sine wave is 3 dB above the respective root mean square (RMS) value. Stimulus frequencies between 5 and 80 kHz were used, and we typically presented 30 ms pulses with a rise/fall time of 0.3 ms and a repetition rate of 3 Hz.

Neuronal activity was recorded using electrolytically sharpened tungsten hook electrodes. For grounding, a reference electrode was inserted into one of the compound eyes. The signal was amplified and stored together with the stimulus pulses on DAT (Biologic, DTR 1200) for off-line computer analyses. Threshold intensity was defined as a mean response of one spike per stimulus. For threshold determination, the signal intensity was increased stepwise from approximately 35–95 dB SPL in steps of 3 dB. Each stimulus intensity was presented five times, and the mean number of spikes in the response was plotted *versus* the stimulus intensity. The threshold intensity corresponding to a mean spike number of one spike per stimulus was finally extrapolated by fitting fifth-order polynomial functions to the plots. All values are expressed as means ± S.D.

Results

Identification and description of the nerve carrying auditory afference in Acherontia atropos

To identify any head nerve carrying auditory afferent responses in *Acherontia atropos*, we recorded extracellularly

from the proximal regions of the suboesophageal and tritocerebral nerves (mandibular, maxillary, labial and labral nerves, frontal connectives). Auditory afferent responses, which were recognised by their short latencies (5–6 ms at high sound intensities), were only detected in the labral nerves. These are a pair of large nerves arising bilaterally in the anterior region of the tritocerebrum. Since the labral nerves are not mentioned in previous descriptions of the head nervous system of hawkmoths (Eaton, 1974, 1988), their distal branching pattern was analysed using cobalt backfills.

Each labral nerve divides close to the tritocerebrum, with a small side branch running dorsally parallel to the frontal connective and with the main trunk extending anteriorly below the pharynx (the distal branch of this main trunk is shown in Fig. 1). In the anterior region of the pharynx, the main trunk sends a fine side branch to a field of presumably chemosensory sensilla in the ventral cuticle of the pharynx (pharyngeal sensory organ, PSO; Fig. 1A). Further distally, the trunk passes between the anterior edge of the pharynx and the tentorial arm, where it divides into two large branches. One of these branches turns posteriorly, extends below the optic lobes and divides into several branches that innervate the cuticle of the postgena (Fig. 1A). The other branch runs laterally from the pharynx to the dorsal region of the head, where it splits up into a large number of sensory branches (Fig. 1B). Most of these branches extend and terminate between the air sacs covering the upper surface of the pharynx dilator muscles. One branch, however, runs medially in the frontal region of the head, where it innervates a field of presumably chemosensory sensilla located in the epipharyngeal cuticle (epipharyngeal sensory organ, ESO; Fig. 1B). This branch splits off a small side nerve, the pilifer nerve (Fig. 1B), which passes anteriorly between the tentorial processes and enters the labral pilifer (Fig. 1).

Using both the dorsal and the ventral surgical approaches, we identified auditory afferent responses in the proximal region of the labral nerve. When the dorsal approach was used, successful recordings were obtained from two of a total of 17 preparations. In these two preparations, auditory afferent responses were only detected when the nerve had been greatly stretched and when most of the non-auditory background activity had already disappeared. In contrast, auditory afferent responses could be detected easily in almost every preparation (N>20) when the nerves were exposed from the ventral side. In these preparations, we always recorded only from the nerve on the side of the head on which the labial palp remained intact during the dissection, as indicated in Fig. 1A.

Characterisation of the auditory afferent response

In all successful recordings from the labral nerve of *Acherontia atropos*, an acoustic stimulus elicited responses in no more than a single auditory afferent unit. This unit, which is characterised by a very low level of spontaneous activity, responded to acoustic stimuli of various frequencies (Fig. 2A), pulse lengths (Fig. 2A,B) and intensities (Fig. 2C). Slight variations in the amplitude of the auditory spikes during the responses reflect the low signal-to-noise ratio and, despite careful examination, we never detected the recruitment of further auditory units in any of the recordings. Thus, the proximal region of the labral nerve appears to contain the axon of a single auditory sensory cell in *Acherontia atropos*.

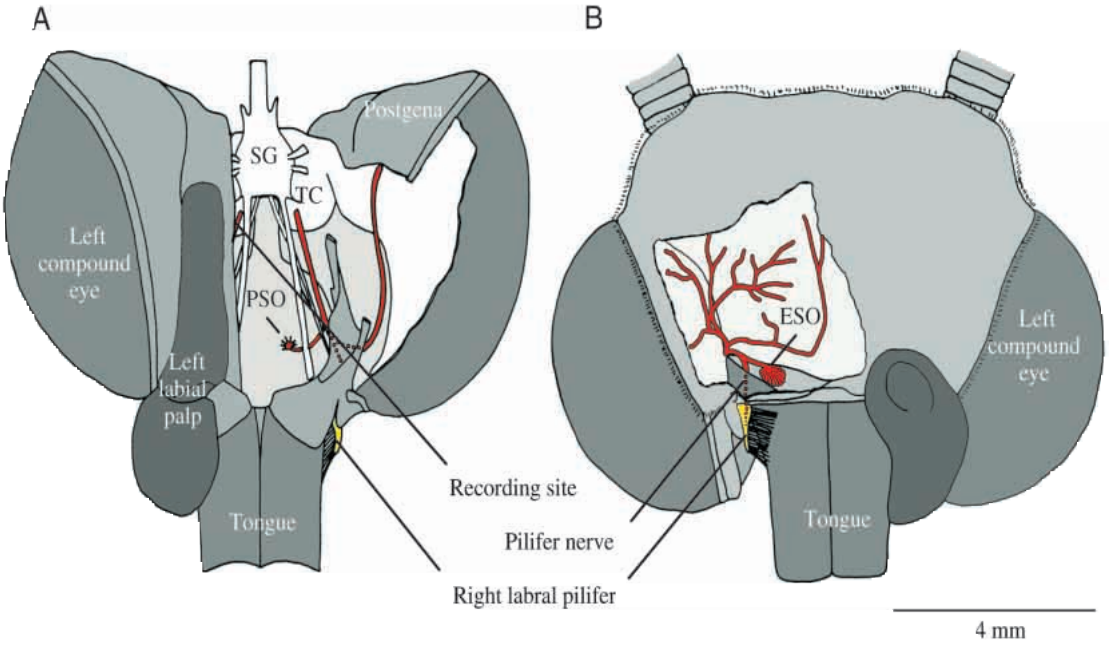

Fig. 1. Distal branching of the labral nerve (red) and innervation of the labral pilifer (yellow) in *Acherontia atropos*. The pilifers are a pair of small bristled appendages of the labrum that are located on either side of the tongue. In the natural arrangement, the pilifers are totally concealed by the labial palps. Here, the right pilifer has been exposed by removal of the right palp. (A) Ventral view of the head with the nerves arising from the suboesophageal ganglion (SG) and the tritocerebrum (TC) exposed. The labral nerve arises from the tritocerebrum, innervates the pharyngeal sensory organ (PSO) in the cuticle of the pharynx and sends main branches to the postgena and to the dorsal region of the head. (B) The same head in frontodorsal view showing the nerve branches that extend in the dorsal region of the head. One branch extends medially in the frontal region of the head innervating an epipharyngeal sensory organ (ESO) and sending a side branch, the pilifer nerve, to the labral pilifer. The region of this nerve from which we recorded from is marked in A.

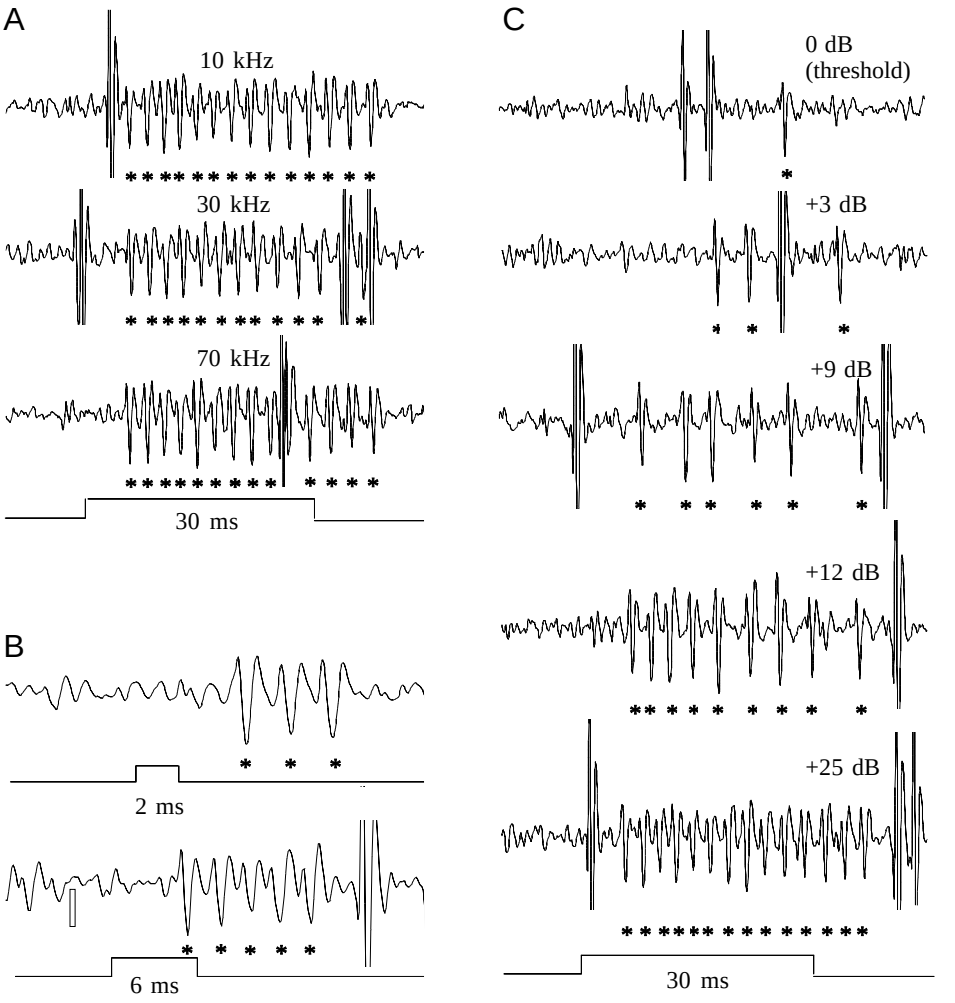

Fig. 2. Afferent responses of the single auditory unit in the labral nerve of *Acherontia atropos*. Auditory spikes are marked by asterisks. (A) Responses elicited by intense stimulation (25–35 dB above threshold) with 30 ms pulses presented at different frequencies. (B) Responses to short 25 kHz pulses of high intensity (92 dB). (C) Responses to 30 ms pulses at 25 kHz and of increasing intensity measured as dB above threshold.

The threshold curves of the auditory sensory cell show best frequencies around 20–25 kHz in all animals studied, with minimum thresholds ranging from 49 to 57 dB SPL (53.2±2.7 dB; eight animals; Fig. 3). The thresholds increase steeply at frequencies below approximately 15–20 kHz, but more slowly towards higher frequencies, and individual Q_{10dB} values, which are measured as the best frequency divided by the frequency bandwidth at the −10 dB points, vary between 0.41 and 0.64 (0.49±0.09; N=8).

Variations in stimulus intensity cause changes in the number of auditory afferent spikes and in the latency of the response. Above threshold intensity, the spike number increases monotonically with increasing stimulus intensity (Figs 2C, 4A). The dynamic range derived from the intensity/response curve (Fig. 4A) is approximately 20 dB, and the slope of the increase calculated from a linear fit to the mean spike number in the intensity range from 0 to +18 dB above threshold is 0.66 spikes dB^{-1} (r^2=0.98, P=0.01). The latency decreases approximately exponentially as the intensity increases above threshold. At threshold intensity, the latency ranges from 17 to 44 ms (28.3±8.0 ms, 25 responses), and it decreases to approximately 5–6 ms at high stimulus intensities (e.g. 5.4±0.6 ms at 35 dB above threshold, n=34 responses; N=8 animals). The dynamic range derived from the latency is approximately 20 dB, which the same range as for the spike number.

The general firing pattern of the auditory afferent unit is phasic–tonic, and the stimulus duration is reflected by both the spike number and the response duration (Fig. 5). Stimuli as short as 2 ms pulses elicit 2–4 afferent spikes (3.1±0.6 spikes, n=32, N=8; Fig. 5A, see also Fig. 2B) if presented at high intensities (25–35 dB above threshold), whereas 1000 ms stimuli elicit trains of approximately 185–240 spikes (211±23 spikes, N=27). The stimulus duration is almost exactly tracked by the response duration. Only if the stimulus duration is shorter than approximately 10 ms does the response duration exceed the stimulus duration (Fig. 5B, see also Fig. 2B). While the spike repetition rate is high during the initial part of the response, with instantaneous rates of approximately 500–550 spikes s^{-1}, it decreases to approximately 160–200 spikes s^{-1} during the later part of the response (Fig. 5C).

Localisation of the auditory sensory organ

To localise the origin of the auditory afferent response in the labral nerve of *Acherontia atropos*, we analysed changes in the

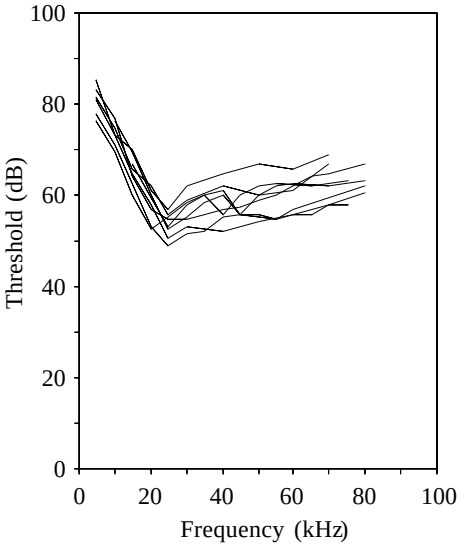

Fig. 3. Individual threshold curves of the auditory afferent response in the labral nerve of *Acherontia atropos* (eight animals).

auditory afferent response caused by ablation experiments. Changes in the response were assessed by measuring intensity/response functions before and after ablation (Fig. 6A). If the labial palp was amputated at its articulation with the cranium, the intensity/response function obtained from the ipsilateral labral nerve shifted by approximately 20 dB to higher intensities. Subsequent amputation of the slender distal half of the ipsilateral pilifer caused only a slight further shift of the curve, but did not totally abolish the afferent response. Auditory afferent activity only disappeared when the basal region of this pilifer was also removed, indicating that the sensory cell had been destroyed.

Neuronal activity inside the pilifer was recorded by inserting an electrolytically sharpened tungsten electrode directly in different regions of the pilifer. Since the ipsilateral palp had to be removed prior to these experiments, 25 kHz pulses of high intensity (90–95 dB SPL) were used for stimulation, thus compensating for the sensitivity loss caused by the removal of the palp. In all three animals studied, auditory afferent responses were detected when the electrode was inserted close to the base of the pilifer, whereas no auditory afferent responses could be recorded from more distal parts of the pilifer. The averaged responses recorded from the basal pilifer region show the same oscillations phase-locked to the stimulus as those in averaged recordings from the proximal region of the labral nerve (Fig. 6B). The latencies of the responses in the pilifer are short (approximately 3.8 ms), indicating that the recording site is close to the auditory sensory organ. The auditory sensory cell must therefore be located in the basal region of the pilifer.

Comparative analyses of sensory structures in the pilifer of hawkmoths

The distribution of sensory structures in the pilifer of hawkmoths was studied using retrograde nickel backfills of the labral nerve in the hearing acherontiine species *Acherontia atropos*, in the non-hearing acherontiine species *Panogena lingens* and in the hearing choerocampine species *Hippotion celerio*. At least 25 successful backfills of the nerve branches inside the pilifer were analysed per species.

In all species studied, the pilifer is innervated by a side branch of the labral nerve, the pilifer nerve, and this nerve enters the pilifer in its proximal region. Inside the pilifer, the nerve extends to the base of the sensory setae covering the medial surface of the pilifer, where it splits into a large number of sensory branches (Figs 7, 8). All these branches innervate sensory cells associated with setae and, despite careful examination, we never detected any further sensory structures inside the pilifer of any of the species studied.

Before entering the pilifer, the pilifer nerve splits off a fine side branch (Figs 7, 8). This branch extends to the basal pilifer region and terminates in a chordotonal organ that attaches to the base of the pilifer. This chordotonal organ is the only sensory structure in the basal pilifer region and it consists of a single scolopidium and a single sensory cell in all species studied (Fig. 8). The axon and the soma of the sensory cell were often concealed by the pilifer nerve, but the single dendrite, the scolopale cap and the rods of the single scolopidium were intensely stained in most preparations. The length of the scolopale measured from the distal end of the cap to the proximal end of the rod is approximately 10–12 μm in

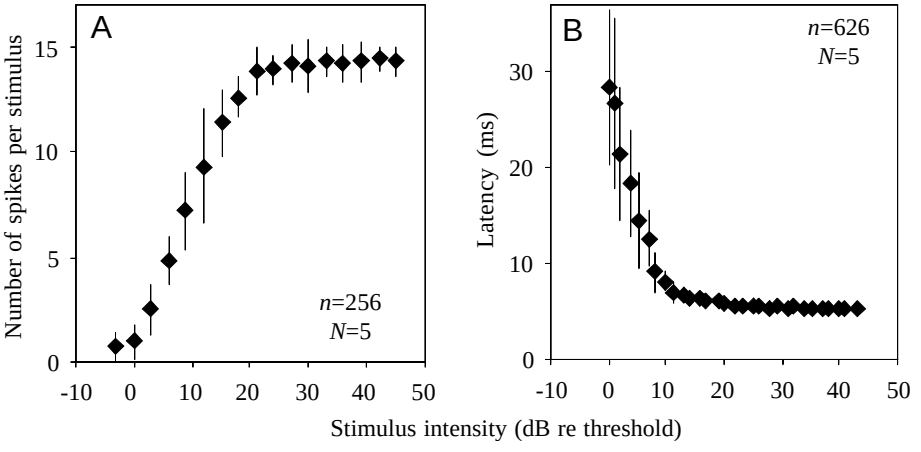

Fig. 4. Changes in the auditory afferent response in *Acherontia atropos* with varying stimulus intensities. (A) Intensity/response curve showing the change in the mean spike number with stimulus intensity. (B) Change in the mean latency with stimulus intensity. Stimuli were 30 ms pulses at 25 kHz; intensities are given relative to the threshold intensity. Values are means ± S.D. '*n*' refers to the number of responses analysed and '*N*' refers to the number of animals studied.

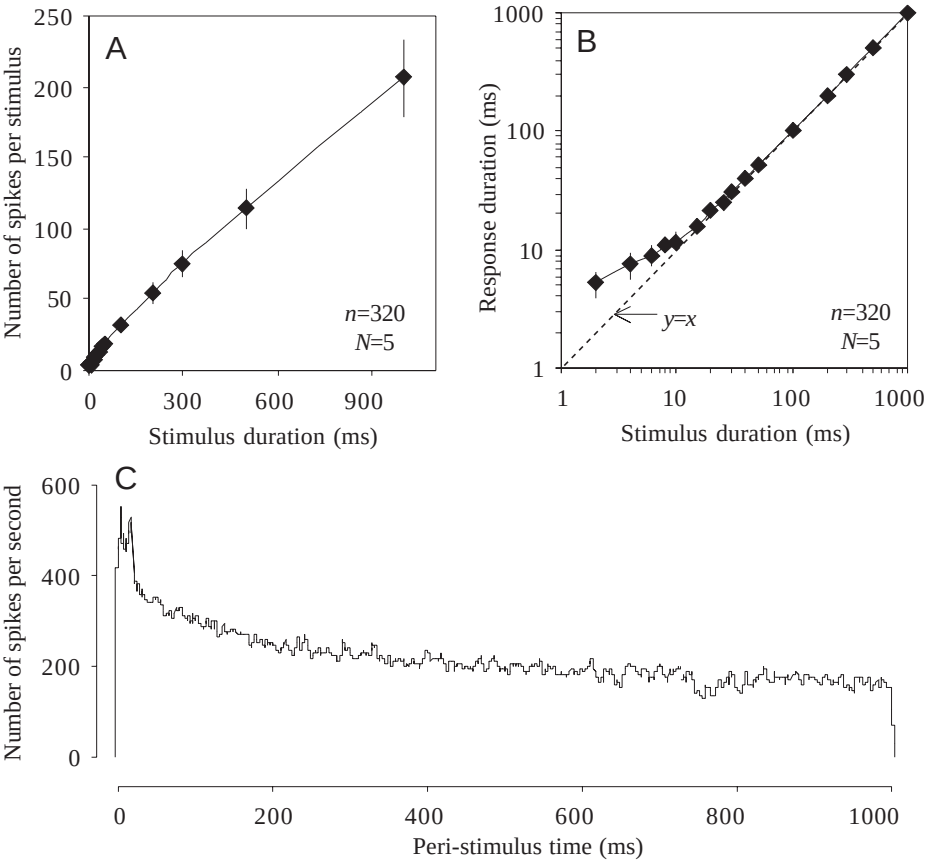

Fig. 5. Changes in the auditory afferent response in *Acherontia atropos* with varying stimulus duration. (A) Mean spike number plotted *versus* stimulus duration. (B) Mean response duration plotted *versus* stimulus duration. Values in A and B are means ± S.D. '*n*' refers to the number of responses analysed and '*N*' refers to the number of animals studied. (C) Changes in the instantaneous spike frequency during stimulation with a 1000 ms pulse. The stimuli were 25 kHz pulses of high intensity (25–35 dB above threshold).

Acherontia atropos and *Hippotion celerio* and approximately 20 μm in *Panogena lingens* (*N*=3 per species). In *Panogena lingens*, the whole chordotonal organ is surrounded by a wide strand of tissue that attaches to the ventral side of the basal pilifer region. A comparable tissue strand was never detected in any preparation from *Acherontia atropos* and *Hippotion celerio*, but in these species an attachment cell connecting to the proximal edge of the pilifer was visible in some preparations (e.g. the preparation from *Acherontia atropos* shown in Fig. 8A).

Discussion

Homology of the auditory sensory organs in acherontiine and choerocampine hawkmoths

Pilifer palp hearing organs have evolved independently in hawkmoths of the distantly related Acherontiina and Choerocampina (Roeder et al., 1968; Göpfert and Wasserthal, 1999). Nevertheless, the hearing organs found in both subfamilies are made up not only of homologous mouthparts but also of homologous sensory structures.

In the hearing acherontiine hawkmoth species *Acherontia atropos*, the proximal region of the labral nerve was shown to carry the afferent responses of a single auditory sensory cell. This cell must be located in the basal pilifer region, as shown by ablation experiments and by recordings of neuronal activity in different regions of the pilifer. The only sensory structure in the basal pilifer region is a chordotonal organ, which attaches to the base of the pilifer. All other sensory cells inside the pilifer are associated with sensory setae, and these setae have previously been shown not to be involved in sound perception (Göpfert and Wasserthal, 1999). The auditory function of the chordotonal organ is further supported by its structure: in agreement with our neurophysiological data, this organ consists of a single scolopidium with a single sensory cell.

These results in a hearing acherontiine hawkmoth species obviously agree with the results of previous neurophysiological studies indicating the presence of a single auditory sensory cell in the pilifer of Choerocampina (Roeder and Treat, 1970; Roeder, 1972). Since a chordotonal organ consisting of a single sensory cell is the only sensory structure in the pilifer that might serve an auditory function in hearing Acherontiina and Choerocampina, homologous pilifer chordotonal organs appear to serve as acoustic receptors in both hawkmoth taxa. Structural similarities supporting the homology of the pilifer chordotonal organs of Acherontiina and Choerocampina are: (1) the absence of other sensory structures in the basal region of the pilifer; (2) its attachment to the base of the pilifer; (3) the presence of a single scolopidium and a single sensory cell; and (4) innervation by similar branches of the labral nerve. The homology of the auditory sensory organs of Acherontiina and Choerocampina is further supported by the existence of a

similar chordotonal organ in non-hearing hawkmoths, as will be discussed below.

Structural and physiological characteristics – comparisons

Although the ears of hawkmoths are unique in being formed by the mouthparts, the auditory sensory structures themselves are similar to those found in other insects with respect both to their structure and to their physiology.

In hearing acherontiine and choerocampine hawkmoths, the auditory sensory organs are chordotonal organs which consist of a single scolopidium. This scolopidium can be characterised with respect to its structure as being mononematic, i.e. having a scolopale cap and being monodynal, i.e. consisting of a single sensory cell (compare Field and Matheson, 1998). This type of insect sensory organ appears to be best suited for the transduction of ultrasonic sounds. In all tympanate insects studied to date, the auditory sensory organs are chordotonal organs, and the auditory sensory organs of tympanate insects are always made up of scolopidia that are both mononematic and monodynal (Yack and Roots, 1992; for a review, see Field and Matheson, 1998).

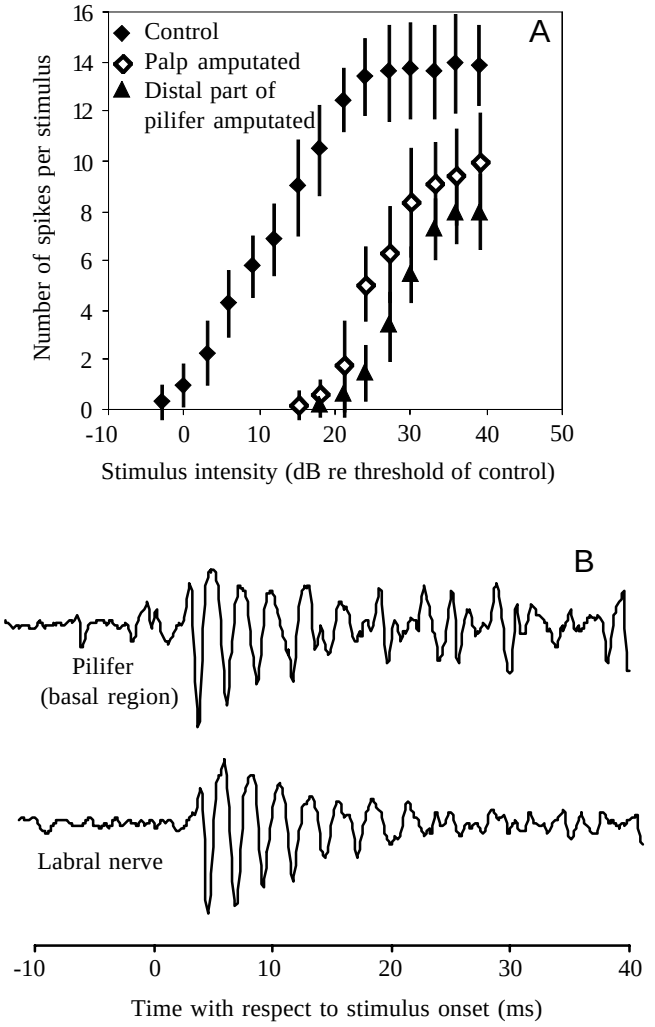

Fig. 6. Origin of the auditory afferent response in the labral nerve of *Acherontia atropos*. (A) Changes in the intensity/response curve caused by amputation of the labial palp and subsequent amputation of the distal half of the pilifer. The auditory afferent response only disappeared if the basal parts of the pilifer were also amputated. Values are means ± S.D. (*N*=3). (B) Averaged recordings of auditory afferent activity in the basal region of the pilifer and in the proximal region of the labral nerve. Stimulation was at a frequency of 25 kHz and at a sound intensity of 93 dB SPL.

Ears as comparatively simple as those in hawkmoths have hitherto only been detected in other nocturnal Lepidoptera. These insects presumably evolved hearing in response to bat predation. The frequency sensitivity of moth ears usually corresponds to the ultrasonic frequencies that dominate the echolocation signals of insectivorous bats, and the auditory input triggers evasive manoeuvres (for reviews, see Spangler, 1988; Surlykke, 1988). For this behavioural task, information about the presence or distance of an approaching bat is sufficient, and small numbers of sensory cells seem to be sufficient to provide this information. Four auditory sensory cells are found in the ears of geometrid, pyraloid and drepanid moths, and only two in those of noctuid and uraniid moths (Sick, 1937; Roeder and Treat, 1957; Surlykke and Filskov, 1997). Furthermore, the ears of notodontid moths have been shown to contain only a single auditory sensory cell (Surlykke, 1984).

The ears of hawkmoths fit well into this general pattern, not only with respect to the presence of a single sensory cell but also with respect to their physiological response characteristics: like the the auditory sensory cells of noctuid, notodontid and geometrid moths (Surlykke and Miller, 1982; Surlykke, 1984; Surlykke and Filskov, 1997), the cell is sensitive almost exclusively to ultrasonic frequencies. In addition to similar frequency characteristics, similar intensity characteristics (with maximum sensitivities around 50–55 dB and dynamic ranges of approximately 20 dB, as found in *Acherontia atropos*) have also been reported from the single sensory cell of notodontids (Surlykke, 1984) and the more sensitive sensory cell (A1 cell) of nocutids (Surlykke and Miller, 1982). These similar response characteristics of the auditory sensory cells of different groups of moths indicate that, despite the different construction of the ears, a common selective pressure led to similar physiological adaptations. Behavioural studies of acoustic startle responses have demonstrated that hawkmoths, like other moths, use hearing in the context of bat avoidance (Göpfert and Wasserthal, 1999). Our studies also confirm that even in *Acherontia atropos*, which is well known for producing sounds with the pharynx, hearing is unlikely to be used in the context of intraspecific communication: the sounds produced by this species have a harmonic structure with most energy at frequencies below 20 kHz (Busnel and Dumortier, 1960; Sales and Pye, 1974) and do not obviously match the frequency sensitivity of the auditory sensory cell.

Evolutionary precursors of the auditory sensory organs in hawkmoths

Insect hearing organs are generally assumed to be phylogenetically derived from proprioceptive chordotonal

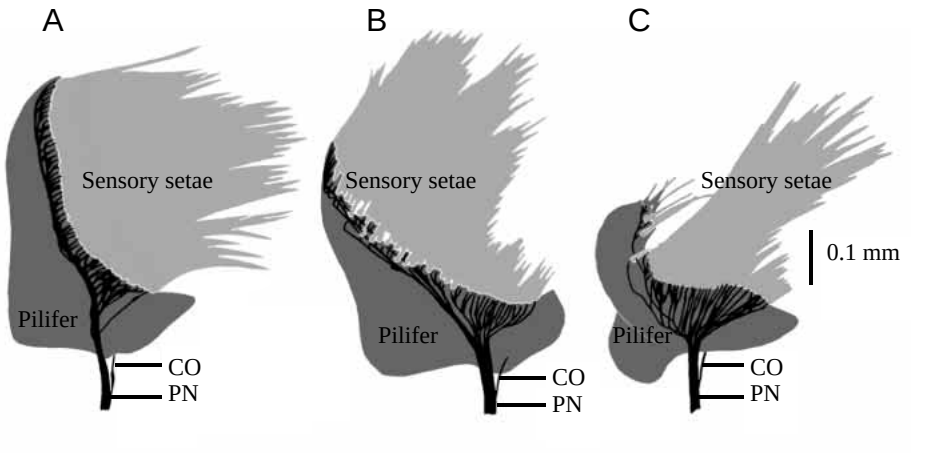

Fig. 7. Schematic drawings of pilifer innervation in hawkmoths derived from nickel backfills. (A) Hearing acherontiine species (*Acherontia atropos*); (B) non-hearing acherontiine species (*Panogena lingens*); (C) hearing choerocampine species (*Hippotion celerio*). PN, pilifer nerve; CO, chordotonal organ.

organs. Chordotonal organs usually serve proprioceptive functions, and the auditory sensory organs of insects appear to be an evolutionary specialisation of the basic proprioceptive chordotonal organ (for reviews, see Boyan, 1993; Field and Matheson, 1998).

We have identified the presumptive homologue of the auditory sensory organs of hearing hawkmoths in the non-hearing *Panogena lingens*. As in hearing species, a chordotonal organ attaches to the base of the pilifer in this species, and the position of the pilifer chordotonal organ suggests that it serves a proprioceptive function monitoring pilifer movements. Structural similarities suggest homology between the pilifer chordotonal organs of non-hearing and hearing hawkmoths, and the homology can be extended to the cellular level since these organs contain a single sensory cell in all species studied. Thus, hearing in choerocampine and acherontiine hawkmoths appears to have evolved convergently from a single proprioceptive sensory cell. The proprioceptive evolutionary origin of hearing in hawkmoths was suggested by Roeder (1972), who demonstrated that mechanical stimulation of the pilifer elicits interneurone responses in both hearing and non-hearing hawkmoth species. He concluded that ‘the pilifer belongs to and has evolved from a vibration sense modality by developing an especially high sensitivity to external displacement’ (Roeder, 1972). However, why not just one but two subtribes of hawkmoth constructed an ear using the chordotonal organ on the mouthparts, whereas other moths evolved ears sited around the waist, is not known.

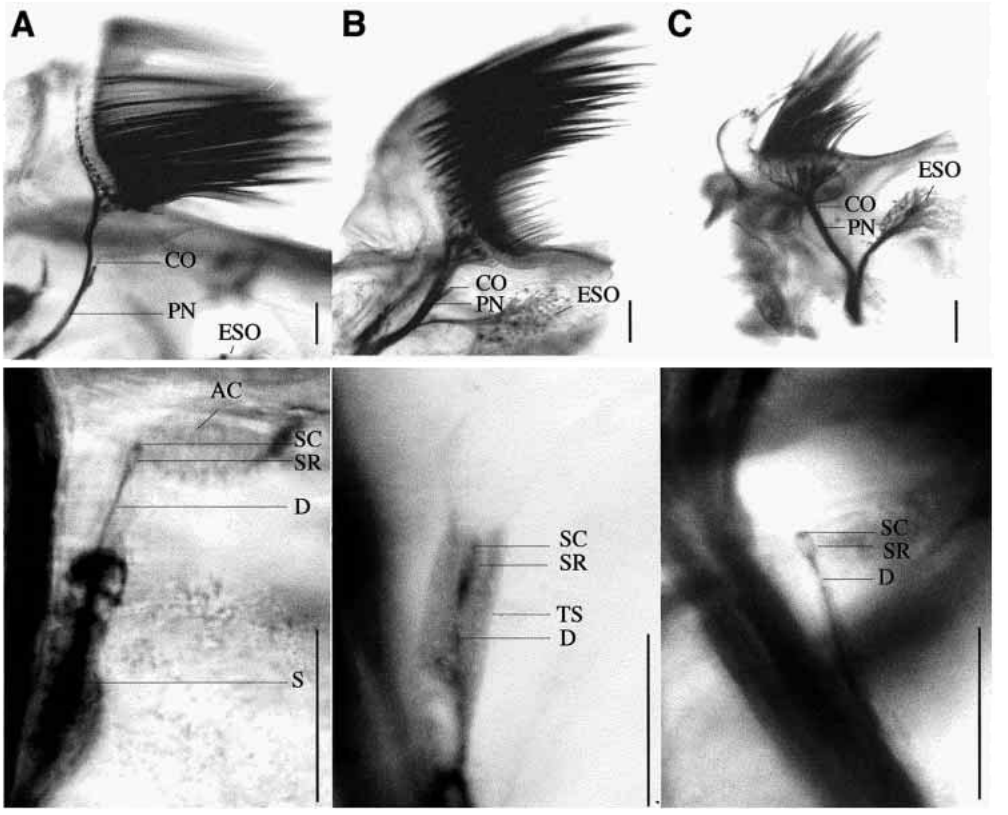

Fig. 8. Branching of the pilifer nerve (PN) revealed by nickel backfills. (A) *Acherontia atropos*; (B) *Panogena lingens*; (C) *Hippotion celerio*. Whole mounts of the pilifer are shown in the upper row, and details of the chordotonal organ at the base of the pilifer are shown in the lower row. AC, attachment cell; CO, chordotonal organ; D, dendrite; ESO, epipharyngeal sensory organ; PN, pilifer nerve; S, sensory cell; SC, scolopale cap; SR, scolopale rod; TS, tissue strand. Scale bars, 100 μm (upper row) and 50 μm (lower row).

The functional transition of the pilifer chordotonal organ from proprioception in non-hearing hawkmoth species to audition in hearing moths is accompanied by structural modifications to peripheral structures, the labial palps and the labral pilifers, which appear to increase the acoustic sensitivity of the sensory organ (Roeder et al., 1970; Roeder, 1972; Göpfert and Wasserthal, 1999). Additionally, the functional transition appears to be accompanied by structural modifications to the acoustic receptor organ itself. Although we did not study the morphology of the pilifer chordotonal organs in detail, our neuroanatomical analyses suggest that the lengths of the scolopale and the amount of surrounding tissues are reduced in hearing species of both subtribes. Comparisons between the auditory sensory organs of noctuid moths and their proprioceptive homologues in saturniid moths have revealed similar modifications, including a reduction in the length of the whole organ and a reduction in the amount of surrounding tissues (Yack and Roots, 1992). In contrast to the auditory sensory organs of noctuid moths, however, the simpler ears of hawkmoths offer the opportunity to examine such structural modifications not only on the basis of homologous chordotonal organs but also on the basis of their subunits, the scolopidia. Since the auditory functions of the pilifer chordotonal organ have evolved not once but at least twice independently, hawkmoths will provide an excellent model in which to analyse structural differences between proprioceptive and auditory scolopidia in closely related species.

Many thanks to Annemarie Surlykke for valuable discussions and for critically reading earlier versions of the manuscript and to Claudia Obermeier and Angela Bruns for rearing the animals. This work was supported by the Deutsche Forschungsgemeinschaft (Wa 258/4).

References

Altman, J. S. and Tyrer, N. M. (1980). Filling selected neurons with cobalt through cut axons. In *Neuroanatomical Techniques* (ed. N. J. Strausfeld and T. A. Miller), pp. 377–402. London, New York, Tokyo: Springer.

Boyan, G. S. (1993). Another look at insect audition: the tympanic receptors as an evolutionary specialization of the chordotonal system. *J. Insect Physiol.* **39**, 187–200.

Busnel, R. G. and Dumortier, B. (1960). Vérification par des méthodes d'analyse acoustique des hypothèses sur l'origine du cri du Sphinx *Acherontia atropos* L. *Bull. Soc. Ent. Fr*. **64**, 44–58.

Eaton, J. L. (1974). Nervous system of the head and thorax of the adult tobacco hornworm *Manduca sexta* (Lepidoptera: Sphingidae). *Int. J. Insect Morph. Embryol.* **3**, 47–66.

Eaton, J. L. (1988). *Lepidopteran Anatomy*. New York, Chichester, Brisbane, Toronto, Singapore: John Wiley & Sons.

Field, L. H. and Matheson, T. (1998). Chordotonal organs of insects. *Adv. Insect Physiol.* **27**, 1–228.

Göpfert, M. C. and Wasserthal, L. T. (1999). Hearing with the mouthparts: behavioural responses and structural basis of ultrasound perception in acherontiine hawkmoths. *J. Exp. Biol*. **202**, 909–918.

Kaissling, K. G. and Thorson, J. (1980). Insect olfactory sensilla: Structural aspects of the functional organization. In *Receptors for Neurotransmitters, Hormones and Pheromones in Insects* (ed. D. B. Sattelle, L. M. Hall and J. G. Hildebrand), pp. 261–282. Amsterdam, New York, Oxford: Elsevier.

Quirke, D. J. and Brace, R. C. (1979). Differential staining of cobalt and nickel-filled neurons using rubeanic acid. *J. Microsc.* **115**, 161–163.

Roeder, K. D. (1972). Acoustic and mechanical sensitivity of the distal lobe of the pilifer in choerocampine hawkmoths. *J. Insect Physiol.* **18**, 1249–1264.

Roeder, K. D. (1976). Joys and frustrations of doing research. *Persp. Biol. Med*. **19**, 231–245.

Roeder, K. D. and Treat, A. E. (1957). Ultrasonic reception by the tympanic organ of noctuid moths. *J. Exp. Zool.* **134**, 127–157.

Roeder, K. D. and Treat, A. E. (1970). An acoustic sense in some hawkmoths (Choerocampinae). *J. Insect Physiol.* **16**, 1069–1086.

Roeder, K. D., Treat, A. E. and Vande Berg, J. S. (1968). Auditory sense in certain hawkmoths. *Science* **159**, 331–333.

Roeder, K. D., Treat, A. E. and Vande Berg, J. S. (1970). Distal lobe of the pilifer: an ultrasonic receptor in choerocampine hawkmoths. *Science* **170**, 1098–1099.

Sales, G. and Pye, J. D. (1974). *Ultrasonic Communication by Animals*. London: Chapman & Hall.

Sick, H. (1937). Die Tympanalorgane der Uraniden und Epiplemiden. *Zool. Jahrb. Anat.* **63**, 352–398.

Spangler, H. G. (1988). Moth hearing, defense and communication. *A. Rev. Ent.* **33**, 59–81.

Surlykke, A. (1984). Hearing in notodontid moths: a tympanic organ with a single auditory neurone. *J. Exp. Biol.* **113**, 323–335.

Surlykke, A. (1988). Interactions between bats and their prey. In *Animal Sonar* (ed. P. E. Nachtigall and P. W. B. Moore), pp. 551–655. New York, London: Plenum Press.

Surlykke, A. and Filskov, M. (1997). Hearing in geometrid moths. *Naturwissenschaften* **84**, 356–359.

Surlykke, A. and Miller, L. A. (1982). Central branchings of three sensory axons from a moth ear (*Agrotis segetum*, Noctuidae). *J. Insect Physiol.* **18**, 357–364.

Yack, J. E. (1993). Janus Green B as a rapid, vital stain for peripheral nerves and chordotonal organs in insects. *J. Neurosci. Meth.* **49**, 17–22.

Yack, J. E. and Roots, B. I. (1992). The metathoracic wing-hinge chordotonal organ of an atympanate moth, *Actias luna* (Lepidoptera, Saturniidae): A light- and electron-microscopic study. *Cell Tissue Res*. **267**, 455–471.