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HEARING WITH THE MOUTHPARTS: BEHAVIOURAL RESPONSES AND THE STRUCTURAL BASIS OF ULTRASOUND PERCEPTION IN ACHERONTIINE HAWKMOTHS

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Summary

In contrast to previous assumptions, mouthparts form hearing organs not only in choerocampine hawkmoths but also in some distantly related acherontiine hawkmoth species. Four of the six acherontiine species studied revealed responses to ultrasonic sounds when stimulated during tethered flight. The responses included changes in flight speed and non-directional turns. Individuals from two species also responded by emitting sound. The minimum thresholds of the flight pattern changes were approximately 70 dB in all species studied, with species-specific best frequencies between 30 and 70 kHz. Some acherontiine species also move their tongue in a stereotyped way when stimulated acoustically. The activity of the muscles involved in this tongue reflex was characterized in the present study and used in combination with ablation experiments to localize the hearing organ. These experiments revealed auditory functions of the labial palps and the labral pilifers similar to those found in Choerocampina. The palp contributes a 20–25 dB rise in sensitivity, whereas the pilifer appears to contain the sensory organ. Structural differences suggest a convergent evolution of hearing in hawkmoths: in the place of the swollen palps of Choerocampina, acherontiine species capable of hearing possess a scale-plate of the palps that interacts with an articulating pilifer, while this modification is absent in closely related non-hearing species.

Key words: acoustic startle response, bat avoidance, hearing organ, evolution, insect, bioacoustics, Sphingidae.

Introduction

A unique mechanism of sound perception is found in hawkmoths of the subtribe Choerocampina (=Choerocampinae *sensu* Rothschild and Jordan, 1903) (Roeder et al., 1968, 1970; Roeder and Treat, 1970; Roeder, 1972; for reviews, see Sales and Pye, 1974; Michelsen and Nocke, 1974; Roeder, 1967; Hutchings and Lewis, 1983; Scoble, 1992). Like many other nocturnal insects, choerocampine hawkmoths are sensitive to the ultrasonic sounds emitted by echolocating bats and probably use hearing in the context of bat avoidance (Roeder et al., 1968). Their hearing organs, however, differ from those of other insects. In the subtribe Choerocampina, hearing organs are located on either side of the head and each organ is made up of two different mouthparts, a labial palp and a labral pilifer (Roeder and Treat, 1970; Roeder et al., 1970).

The acoustic sensitivity of Choerocampina decreases approximately 50- to 100-fold after amputation of the labial palp, whereas no sensitivity remains after amputation of the pilifer. Thus, the labial palp appears to act as an accessory auditory structure increasing the acoustic sensitivity, while the labral pilifer probably carries the auditory sensory cells (Roeder and Treat, 1970; Roeder et al., 1970). The auditory functions of the mouthparts are accompanied by characteristic structural modifications (e.g. Roeder, 1972). In Choerocampina, the second segment of the labial palp is obviously swollen and filled by large air sacs, while the cuticle on the inner surface of this segment is devoid of scales and is thin-walled. In addition, the pilifer is divided into two separate lobes in Choerocampina, and the distal pilifer lobe is in close contact with the inner surface of the second palp segment. This segment is thought to vibrate when it receives acoustic signals, thus stimulating a still unidentified sensory cell in the pilifer by deflecting the distal pilifer lobe (Roeder et al., 1970; Roeder, 1972).

Auditory functions of the pilifer–palp system are thought to be restricted to Choerocampina. The swollen second palp segment and the bilobed pilifer, i.e. those structural modifications that seem to be essentially related to the auditory function of the system, are both apomorphic characters of the subfamily (for a review, see Scoble, 1992). The restricted distribution of this hearing organ is further supported by comparative studies. Roeder and Treat (1970) and Roeder (1972) examined the acoustic sensitivity of

approximately 20 non-choerocampine hawkmoth species of different subfamilies by investigating auditory interneurone responses in the neck connectives. Such responses were only detected in two sesiine species, and in both of these the pilifer–palp system was shown not to be involved in sound perception. Although Roeder and Treat (1970) concluded that some non-choerocampine species may have evolved hearing organs in other body regions, such organs have yet to be localized.

The hawkmoth subtribe Acherontiina (=Acherontiinae *sensu* Rothschild and Jordan, 1903) comprises some of the largest hawkmoth species, including the death's head hawkmoth *Acherontia atropos*. Resting animals of this species respond to acoustic stimulation with interruptions of the regular abdominal ventilatory movements and changes in heart activity, whereas active animals arrest shivering and change their flight pattern (Wasserthal, 1996, and in preparation). These observations gave rise to a systematic analysis of the acoustic sensitivity of, and the mechanism of sound perception by, acherontiine hawkmoths. In the present study, we examine the ultrasonic sensitivity of several acherontiine hawkmoth species and analyze the structural basis of sound perception. To study the acoustic sensitivity, we used a behavioural approach focusing on acoustically elicited changes in the flight pattern of tethered flying animals and on a newly discovered tongue reflex that is described and characterised. The activity of the muscles involved in the tongue reflex is used in combination with ablation experiments to localize the hearing organ. The response characteristics and the structure of the auditory sensory organ itself and the functional mechanism of sound perception in hearing Acherontiina and Choerocampina will be described and compared in subsequent publications.

Materials and methods

Animals

Animals of six acherontiine and two choerocampine hawkmoth species were studied: *Acherontia atropos* L., *Coelonia mauritii* Butler, *Coelonia solani* Boisduval, *Xanthopan morgani* Walker, *Panogena lingens* Butler and *Panogena jasmini* Boisduval of the Acherontiina, and *Euchloron megaera* L. and *Hippotion celerio* L. of the Choerocampina. All animals were raised in the laboratory from stocks originating from Madagascar (all acherontiine species and *Euchloron megaera*), Kenya (some *Acherontia atropos*) and the Canary Islands (some *Acherontia atropos*, *Hippotion celerio*). The adults of most species were kept in flight cages (2 m×2 m×2 m) in temperature-controlled conditions at approximately 25 °C and 80 % relative humidity on a natural light cycle. The adults of *Acherontia atropos* were kept in boxes at 18 °C with continuous illumination to minimize activity. In the flight cages, the animals were provided with a 15 % honey solution offered in artificial flowers, with the exception of *Acherontia atropos* which were fed manually twice a week with a 50 % honey solution.

Morphology

The external morphology of the labial palps and the labral pilifers was studied in freshly killed animals. To expose the contact region between the palps and the pilifers, the heads were split in the median axis, and the tongue was removed in its basal region. For documentation, we used a Wild M400 photomacroscope connected to a Wild MPS500 controlling unit. Some of the preparations were air-dried, sputtered with gold, and examined using a Hitachi S800 scanning electron microscope. During air-drying, insect pins were used to fix the palps in their natural position, thus preventing *post mortem* changes in the natural arrangement. In some of the ablation experiments, the labial nerves (ON3 and ON4; for nomenclature and description, see Eaton, 1988) innervating the labial palps were severed on either side of the suboesophageal ganglion. For this treatment, the suboesophageal ganglion was exposed from the ventral side by removing the labial cuticle between the articulations of the labial palps.

Stimulus generation

All experiments were carried out with the animals in the centre of a 1 m×1 m×1 m Faraday cage lined with sound-absorbing foam to minimize acoustic reflections. The temperature during testing was 20 °C.

The acoustic stimuli were pure tones synthesized by a frequency generator (Voltcraft, model FG 506). The output signal was passed through a digitally controlled attenuator, and the signal intensity and duration were controlled by a computer. The shaped pulses were either fed to an active ultrasound advice loudspeaker system (for stimulation of tethered flying animals) or, after power amplification, to a leaf tweeter (Technics 10TH400C). The speaker was positioned 0.4 m from the animal either with the body axis of the animal being perpendicular to the speaker (for stimulation of tethered flying animals) or with the head of the animal pointing towards the speaker (in all other experiments). The output of the speaker was calibrated with the holder in position using a Bruel and Kjaer 4135 condenser microphone (grid off) and a Bruel and Kjaer 2331 sound level meter. All intensities were determined using the peak-hold function of the sound level meter and are given in dB peak SPL (re 2×10^{-5} Pa).

To stimulate tethered flying animals, trains of 100 pulses 2 ms in duration and separated by 2 ms intervals were presented at frequencies between 10 and 100 kHz. In each trial, the frequency was held constant, and the intensity of the stimulus trains was increased from 50 or 60 dB to 100 dB in steps of either 5 dB (for threshold determination) or 10 dB (in all other experiments) with 5 s intervals between the onsets of subsequent stimulus trains. Subsequent trials were separated by silent periods of at least 5 min to minimize habituation. To characterize acoustically elicited tongue movements, we typically used 30 ms or 100 ms stimuli at frequencies between 3 and 80 kHz and of various intensities presented at a rate of 1 Hz.

Recording and analysis of behavioural responses

Changes in the flight pattern in response to acoustic stimulation were studied in tethered flying hawkmoths. The animals were briefly anaesthetized with CO_2, and their pronotum was fixed to a pin. The distal end of the pin was connected *via* a hinge to a holder. The hinge reduced the freedom of movement of the animal along the anterior–posterior axis. Relative movements of the hinge caused by the thrust of the flying animals were measured using a strain gauge. Although the hinge prevented the animals from making lateral turns, lateral turning tendencies were measured using a differential anemometer consisting of two heated thermistors positioned behind the wings on both sides of the body (modified after Roeder, 1966).

To study acoustically elicited tongue movements, the animals were briefly anaesthetized, and the wings and legs were removed. The animals were glued to a metal holder with the dorsum down and the head protruding over the holder. The head was fixed in position by waxing the compound eyes to the thorax. To expose the dorsal surface of the head, the holder was turned prior to the experiments. Movements of the proboscis were measured using an optoelectronic reflex barrier (Conrad Electronics, model SFH 900) positioned approximately 5 mm in front of the head. For electromyographic (EMG) recordings, a wire electrode was inserted from the dorsal side into the elevator muscle of one of the two galeal lobes forming the tongue (for descriptions of the galeal elevator muscles, see Eaton, 1988). The position of the electrode was checked in several preparations following the experiments.

All signals were amplified using custom-built electronics and stored together with the stimulus pulses on digital audio tape (Bio Logic, model DTR 1200) prior to offline computer analysis. The analyses were performed using the software Turbolab (Stemmer) and Neurolab (B. Hedwig and M. Knepper). Response thresholds of tethered flying animals were arbitrarily defined as the lowest sound pressure level eliciting obvious responses in at least three consecutive trials, whereas the threshold criterion used for muscle activity was the minimum sound pressure level that elicited a mean response of at least one spike per stimulus in five consecutive stimulations. All values are expressed as means ± standard deviations (S.D.).

Results

Morphology

In hawkmoths, the labial palps are normally closely apposed to the head on either side of the tongue with the inner surfaces of their second segments facing the labral pilifers (Fig. 1). The morphology of this inner palp surface and of the labral pilifers differs between Acherontiina and Choerocampina. In Acherontiina, the pilifer is not divided in two distinct lobes, and the second palp segment is neither swollen nor thin-walled on its inner surface, as is characteristic of Choerocampina (Fig. 2C). With respect to the morphology of the pilifer–palp system, the acherontiine species studied divide in two subgroups characterized by the presence or absence of structural modifications.

The first group comprises the *Acherontia*, *Coelonia* and *Xanthopan* species studied. In all these species, the pilifer is obviously slender and prolonged. The cuticle of the pilifer is stiff, and only its medial surface is covered by sensory setae (Fig. 2). A fold runs along the ventral side of the basal pilifer region so that the whole pilifer can easily be deflected in the vertical plane (Fig. 1B). The most obvious characteristic of the group is the second segment of the labial palp, which has a deep depression on its inner surface (Fig. 2A). The proximal half of this depression is roofed over by series of long, overlapping scales forming a plate-like structure (scale-plate; Figs 1B, 2A). In *Acherontia atropos*, the maximum length of these scales is approximately 1.0–1.2 mm, and the total area of the scale-plate is approximately 0.7–1.0 mm^2 (N=4). The scale-plate is the only palp structure that is in contact with the pilifer, and its upper region is closely apposed to the upper surface of the prolonged pilifer (Fig. 1B).

The second group comprises the two *Panogena* species

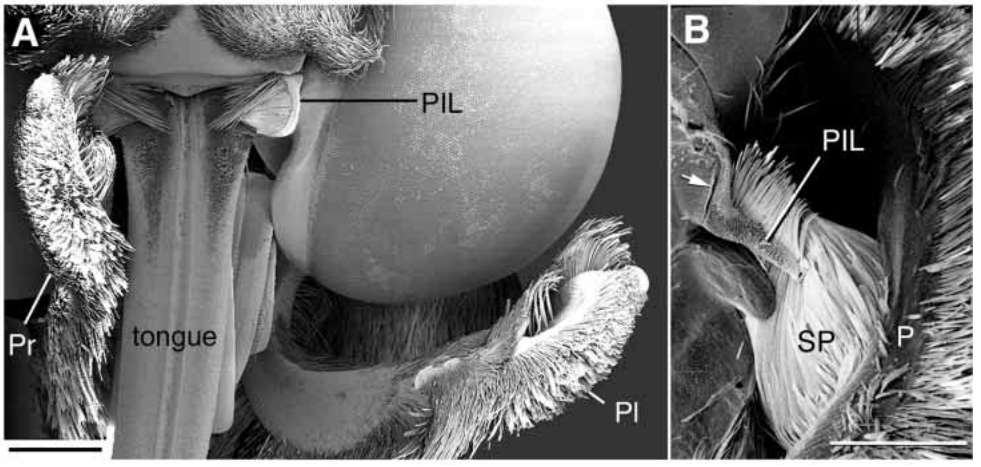

Fig. 1. Scanning electron micrographs of (A) a fronto-dorsal view of the head of *Coelonia mauritii* showing the arrangement of the labial palps and the labral pilifers. The right palp (Pr) is in its natural adducted position concealing the right pilifer, whereas the left palp (Pl) has been deflected laterally from the head, exposing the left pilifer (PIL). (B) Contact region between the pilifers and palps in *Acherontia atropos* viewed from a medio-ventral direction. The inner surface of the second palp segment (P) has a deep depression that is roofed over by long scales, forming a plate-like structure. This scale-plate (SP) is closely apposed to the pilifer (PIL), which can be easily deflected because of a fold running along its basal region (arrow). Scale bars, 1 mm.

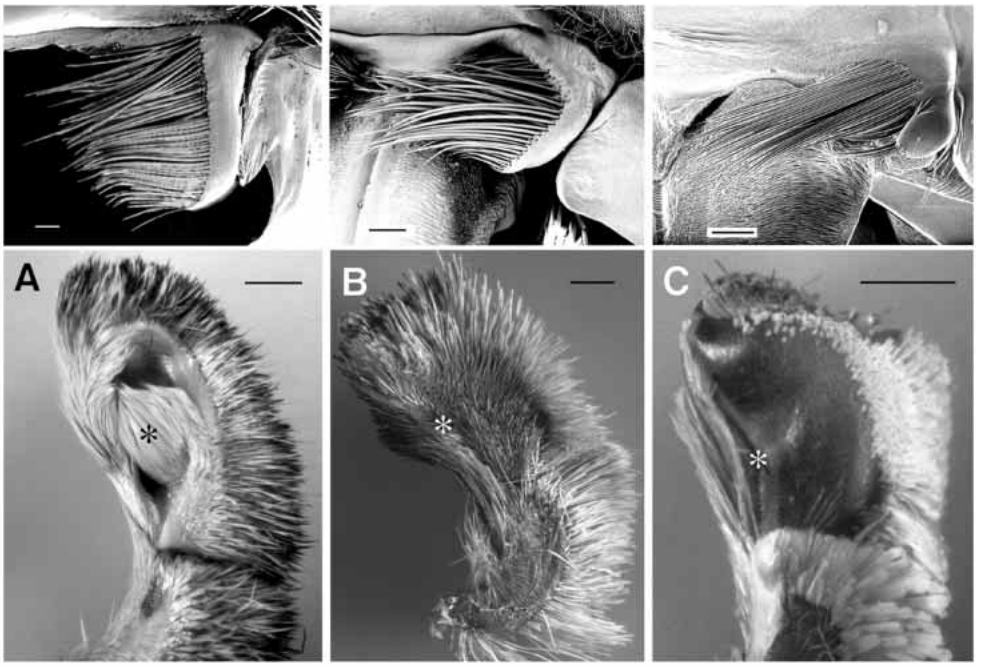

Fig. 2. Morphology of the labral pilifer (upper row; scanning electron micrographs) and the labial palp (lower row, photomicrographs) in acherontiine (A,B) and choerocampine (C) hawkmoths. The left pilifer is shown in dorsal view, and the inner surface of the left labial palp is viewed from a medial direction. Asterisks mark the region of the palp that normally faces the pilifer. (A) *Acherontia atropos*, (B) *Panogena lingens* and (C) *Hippotion celerio*. Scale bars in upper row, 0.1 mm; in lower row, 0.5 mm.

studied. In both species, the pilifer is broad and its cuticle is transparent and soft. Both the medial surface and the medial region of the upper surface of the pilifer are covered by sensory setae (Fig. 2B), and there is no fold at the base of the pilifer. The inner surface of the second segment of the labial palp is not depressed, and no scale-plate is present. Instead, the inner palp surface is completely covered by short scales and lacks any obvious modification (Fig. 2B). Under normal conditions, the upper region of the second palp segment faces the pilifer, with only loose contact between the two mouthparts.

Responses to acoustic stimuli

Tethered flight

Responses of tethered flying animals were studied in six acherontiine and one choerocampine species. Animals of all species flew strongly on a tether without any windstream for up to several hours. Acoustic stimuli presented at frequencies of 10–100 kHz elicited obvious changes in the flight pattern in all animals of the acherontiine species *Acherontia atropos*, *Coelonia mauritii*, *Coelonia solani* and *Xanthopan morgani* and in the choerocampine species *Euchloron megaera* ($N \geqslant 10$ per species). In contrast, animals of the two acherontiine species *Panogena lingens* ($N=9$) and *Panogena jasmini* ($N=1$) never responded to acoustic stimuli even when stimulated with intensities of up to 110 dB SPL. Thus, acoustically elicited changes in flight pattern were only detected in those acherontiine species that are characterized by structural modifications of the pilifer–palp system.

In all species that responded to acoustic stimulation, the responses included changes in the flight speed, contra- and ipsilateral turning tendencies and the cessation of flight activity (Fig. 3). The changes in the flight pattern were often accompanied by leg extension and by abdominal steering movements. In addition, acoustic stimulation elicited sound production in some individuals of *Acherontia atropos* (14 of 21 animals) and in some male *Xanthopan morgani* (three of five males). These sounds were vocalizations in *Acherontia atropos* and valve stridulation in *Xanthopan morgani*, and were like the sounds produced by animals of both species when handled. Changes in the response with increasing stimulus intensities were examined in *Acherontia atropos* (Fig. 4). The stimulus frequency used in these experiments was 40 kHz. The response probability increased with the stimulus intensity, and stimuli of 100 dB SPL always elicited changes in the flight behaviour. Lateral turning tendencies, often accompanied by

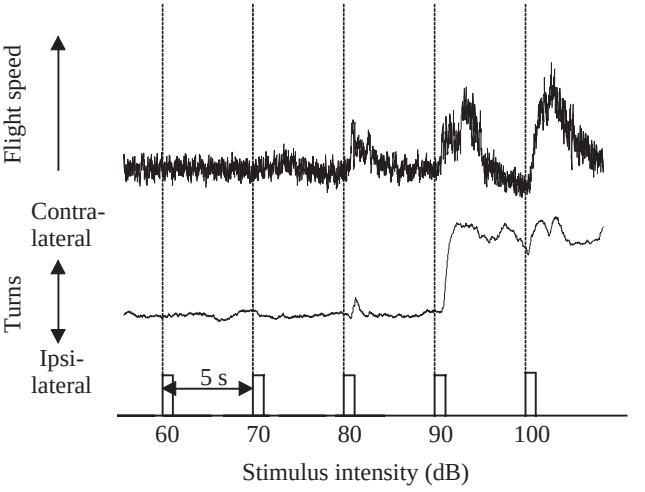

Fig. 3. Representative example data of flight pattern changes elicited by acoustic stimulation in a tethered flying *Acherontia atropos*. Stimuli were trains of 2 ms pulses at 40 kHz frequency. The animal increased its flight speed and tried to turn to the contralateral side when stimulated with intensities of 70–100 dB.

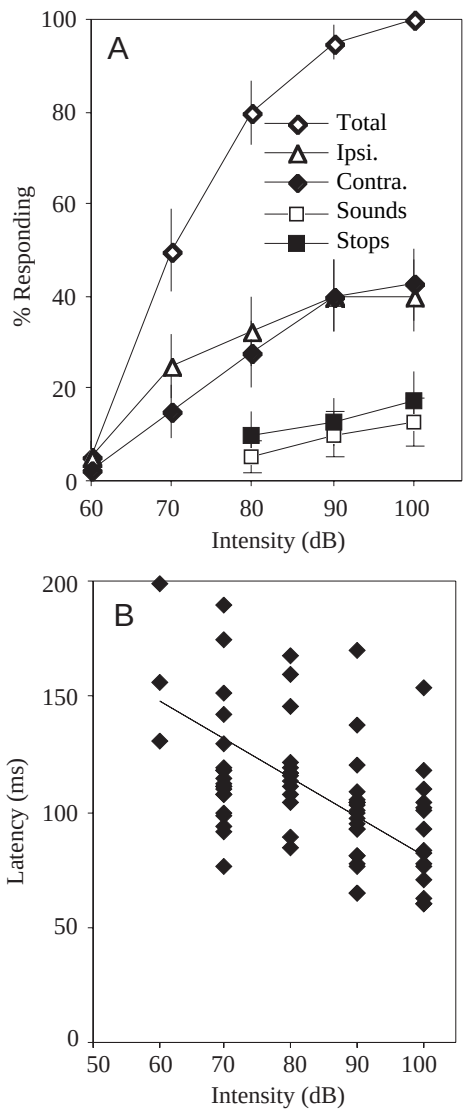

Fig. 4. Changes in the response probability with increasing stimulus intensities in *Acherontia atropos* (40 kHz stimulus frequency). (A) Total response probability as well as probabilities of contra- (Contra) and ipsilateral (Ipsi) turning tendencies, cessation of flight activity (stops) and sound production. Means and standard deviations are calculated from pooled data (200 stimulations) from 10 animals. (B) Latency of turning tendencies occurring in a time window of 200 ms after stimulus onset plotted *versus* the stimulus intensity (81 responses of 10 animals).

changes in flight speed, were detected most often, whereas cessation of flight activity and sound production only occurred in response to high stimulus intensities. The turning tendencies showed no consistent orientation relative to the sound source, and the mean relative frequencies of ipsi- and contralateral turns calculated from pooled data for 10 animals were similar at all stimulus intensities tested (Fig. 4A). Behavioural latencies were determined for lateral turning tendencies occurring in a time window of 200 ms after stimulus onset (Fig. 4B). The response latencies (ms) decreased with increasing stimulus intensities (dB) (linear regression: $y=-1.7x+247$, $r^2=0.52$; $P<0.01$; Spearman rank correlation, two-tailed test). The mean latencies were 118±27 ms at 70 dB SPL ($N=23$) and 89±27 ms at 100 dB SPL ($N=21$). The minimum latency measured was 60 ms.

The threshold curves of the acoustic flight pattern changes cover a wide range of ultrasonic frequencies, with minimum mean thresholds of approximately 70 dB SPL in all species (Fig. 5). The best frequencies are approximately 30 kHz in *Acherontia atropos* (mean threshold 70.7±6.7 dB) and *Coelonia mauritii* (mean threshold 73.7±8.6 dB), and the thresholds increase sharply at frequencies below 20 kHz in both species. In *Coelonia solani* and *Xanthopan morgani*, minimum response thresholds occur at higher frequencies of approximately 60–70 kHz (67.5±8.3 dB for *C. solani* and 75.4±6.9 dB for *Xanthopan morgani*); in the choerocampine *Euchloron megaera*, the best frequency is approximately 50 kHz with a mean threshold of 70.1±6.3 dB.

Tongue movements

Some acherontiine species move their tongue in an obviously stereotyped way when stimulated acoustically. During stimulation, the proboscis is raised in its proximal region and then returned to its starting position after stimulus offset (Fig. 6). Acoustic stimuli elicited this response in all individuals of *Acherontia atropos* and *Coelonia mauritii* ($N>25$ per species), and the movements were observed in both resting and tethered flying animals. In contrast, this response was never detected in animals of the acherontiine species *Panogena lingens* ($N=9$) or the choerocampine species *Hippotion celerio* ($N=7$).

The tongue movements correlate with activity of the galeal elevator muscles in the proximal region of the galeal lobes, as shown by measurements of the movement and simultaneous EMG recordings from different regions of the tongue (Fig. 6C). In addition to this activity in the elevator muscles, acoustically elicited activity in a second muscle was detected in some EMG recordings from *Acherontia atropos*. The activity of this muscle exceeds the stimulus duration and correlates with a slight movement of the two galeal lobes towards one another that was only just visible. The muscle was not identified, but appears to be located proximally towards the base of the tongue, since recordings from that region resulted in the largest spike amplitudes.

The same stimulus elicits uniform movements and elevator muscle activity, even if repeated at high rates (Fig. 6B,C). However, the movements and the muscle activity change if the intensity or the duration of the stimulus pulses are varied (Fig. 7). In both species, *Acherontia atropos* and *Coelonia mauritii*, the number of muscle potentials and the amplitude of the movement increase as the stimulus intensity is increased above threshold. In addition, the amplitude of the muscle potentials recorded in *Coelonia mauritii* increases both with stimulus intensity and during the course of single responses, indicating a recruitment of more units of the muscle that are firing synchronously (Fig. 7B). Since this recruitment of further units precluded meaningful spike count analyses, such

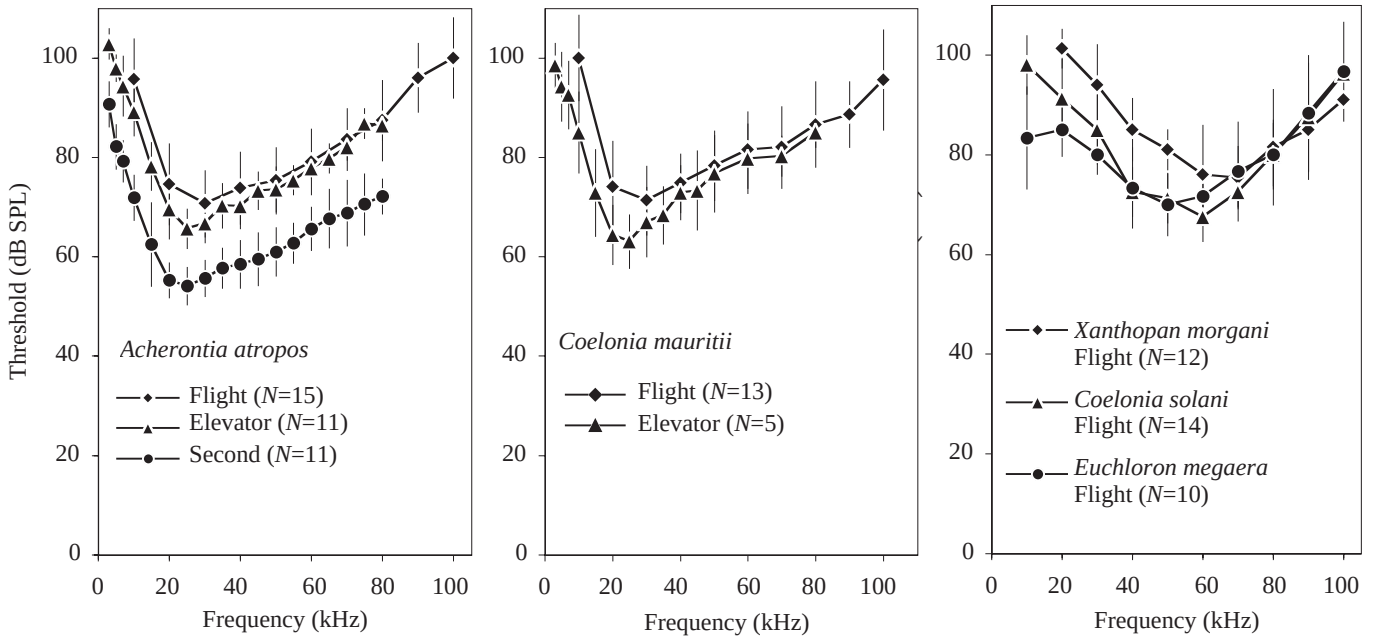

Fig. 5. Mean threshold curves of the flight pattern changes (Flight) and the activity of tongue muscles (Elevator, galeal elevator muscle; Second, unidentified muscle) in Acherontiina (*Acherontia atropos*, *Coelonia mauritii*, *C. solani*, *Xanthopan morgani*) and Choerocampina (*Euchloron megaera*). Values are means ± S.D.

analyses were performed only in *Acherontia atropos*. In this species, the number of muscle potentials increases monotonically with increasing stimulus intensities above threshold (Fig. 8A). The dynamic range is approximately 12 dB if determined from the responses to 30 ms stimuli, but approximately 20 dB if 100 ms stimuli are used. The latency decreases in both *Acherontia atropos* and *Coelonia mauritii* as the intensity is increased above threshold (Fig. 8B). The mean latency 30 dB above threshold is 25.8±3.1 ms (N=47) in *Acherontia atropos* and 20.8±2.0 ms (N=42) in *Coelonia mauritii*. The dynamic ranges derived from the latency of responses to 30 ms stimuli are approximately 20 dB in both species (Fig. 8B). The duration of the movement and the number of muscle potentials per stimulus increase with increasing stimulus duration. Stimulus pulses as short as 5 ms elicit single muscle spikes, whereas long stimuli elicit large numbers of spikes and cause tetanic contractions of the muscle (Figs 7, 8C). The general firing pattern of the muscle is phasic–tonic; the number of muscle potentials increases steeply as the stimulus duration is increased stepwise from 2 ms to approximately 100 ms, but less steeply if the stimulus duration is increased further to up to 1000 ms (Fig. 8C). The slope calculated from a linear fit to the mean spike numbers is 0.09 spikes ms^{-1} at stimulus durations from 2 to 100 ms (r^2=0.96; P<0.001; Spearman rank correlation, two-tailed test), but only 0.04 spikes ms^{-1} at stimulus durations from 100 to 1000 ms (r^2=0.99; P<0.001). The duration of the muscle activity tracks the stimulus duration with a slight decrement within a wide range of durations tested (Figs 7, 8D).

The threshold curves for galeal elevator muscle activity are similar to those of the flight pattern changes in both *Acherontia atropos* and *Coelonia mauritii* (Fig. 5). In both species, the best frequencies are approximately 25 kHz with a mean minimum threshold of 65.6±4.1 dB SPL in *Acherontia atropos* (N=11) and 63.5±5.5 dB SPL in *Coelonia mauritii* (N=5). The activity of the second, unidentified muscle in *Acherontia atropos* indicates a similar frequency-sensitivity, but the threshold curve is shifted by approximately 10 dB to lower intensities with a minimum threshold of 54.1±3.9 dB SPL (N=11).

Ablation experiments

In *Acherontia atropos*, changes in acoustic sensitivity caused by ablation experiments were assessed by comparing the thresholds of the elevator muscle activity before and after ablation. Acoustic stimuli were 100 ms sine-wave pulses presented at frequencies from 5 to 55 kHz.

Transection of the neck connectives did not affect the threshold of muscle activity in any of three animals studied. The acoustic sensitivity also remained unchanged in these animals if the antennae and the distal half of the tongue were subsequently amputated. Only denervation of either the labial palps or the labral pilifers caused obvious changes in the acoustic sensitivity, indicating that both mouthparts are involved in sound perception. The acoustic sensitivity remained unchanged when the labial nerves innervating the labial palps were transected on either side of the suboesophageal ganglion (N=12, Fig. 9A). However, subsequent removal of the scale-plates on the inner surface of both labial palps in the same animals caused a sensitivity loss of approximately 20–25 dB over the whole frequency range studied (mean threshold increase *versus* control at 25 kHz:

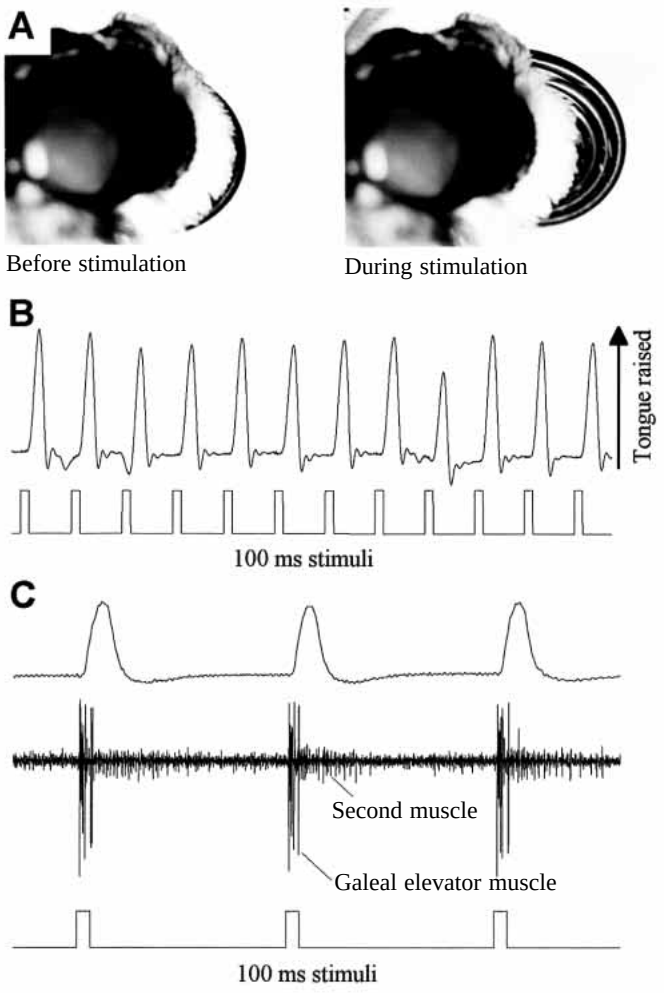

Fig. 6. Tongue movements performed in response to acoustic stimulation. (A) Lateral views of the head of *Coelonia mauritii* before and during acoustic stimulation. During stimulation, the coiled tongue is raised in its proximal region. Stimulation at 25 kHz and 92 dB. (B) Measurement of the tongue movements in *Acherontia atropos* elicited by repeated stimulation with 100 ms pulses at 25 kHz and 92 dB presented at a rate of 1 Hz. (C) Detail from B showing movement traces and the activity of muscles recorded from the proximal region of the tongue. In addition to the large potentials of the galeal elevator muscle, smaller spikes of a second, unidentified muscle are visible.

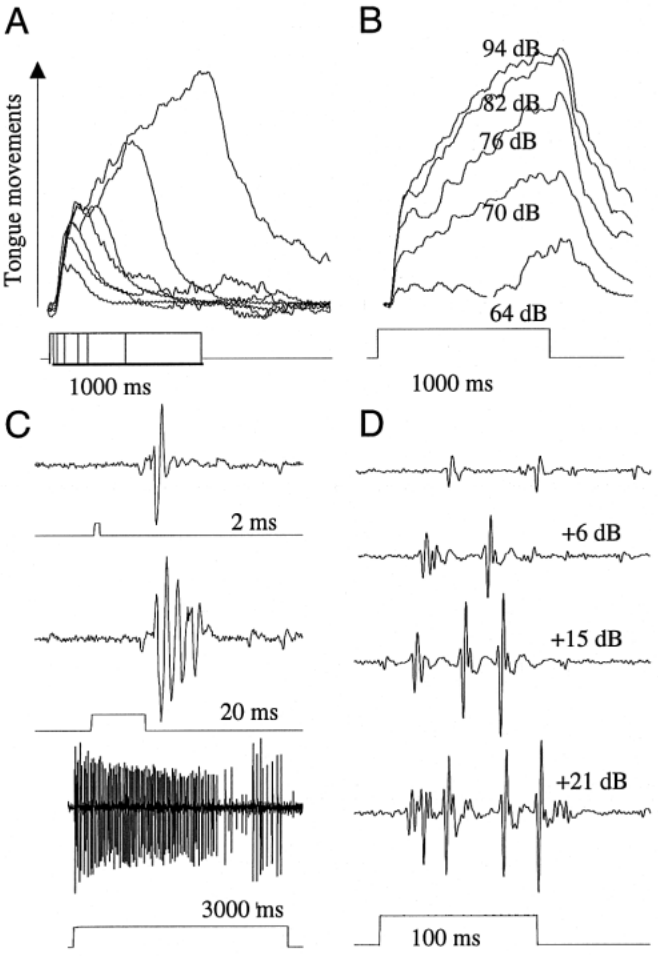

Fig. 7. Changes in the tongue movements with varying stimulus duration (A,C) or intensity (B,D). (A,B) Superimposed traces of the movements elicited in *Coelonia mauritii* by 25 kHz pulses of (A) varying duration but constant intensity (94 dB) and (B) varying intensity but constant duration (1000 ms). (C,D) Variations in galeal elevator muscle activity elicited by stimulation with 25 kHz pulses of (C) varying duration but constant intensity (92 dB) in *Acherontia atropos* and (D) varying intensity but constant duration (100 ms) in *Coelonia mauritii*.

+22.6±3.2 dB, Fig. 9A). No obvious further loss of sensitivity was observed when both palps were subsequently amputated at the articulation of the first and second palp segments (+24.6±2.9 dB at 25 kHz, Fig. 9A). A similar effect was found in other animals, in which both labial palps were deflected laterally from the head (+23.2±3.5 dB at 25 kHz, N=5). However, the effect was reversible in these experiments, and the sensitivity was totally restored when the palps were moved back to the natural position. Removal of the sensory setae of both pilifers did not affect the acoustic sensitivity (N=5, Fig. 9A), but the acoustically elicited muscle activity was eliminated when both pilifers were amputated in their basal regions.

In some additional experiments, only one palp or pilifer was treated. In the example shown (Fig. 9B), a wire electrode was inserted into one of the two galeal lobes, as in the experiments described above, and the stimulus frequency was 25 kHz. At first, only the labial palp contralateral to the galeal lobe from which we recorded was deflected laterally from the head. This treatment did not cause any threshold changes in the muscle response, and the palp was also moved back to the head without affecting the acoustic sensitivity. Subsequently, the palp ipsilateral to the recording site was deflected and the intensity response curve shifted by approximately 20 dB to higher intensities. However, the control level of sensitivity was totally restored when the palp was moved back to the head. A similar, reversible threshold increase was found when both palps were deflected. Amputation of the contralateral pilifer did not affect

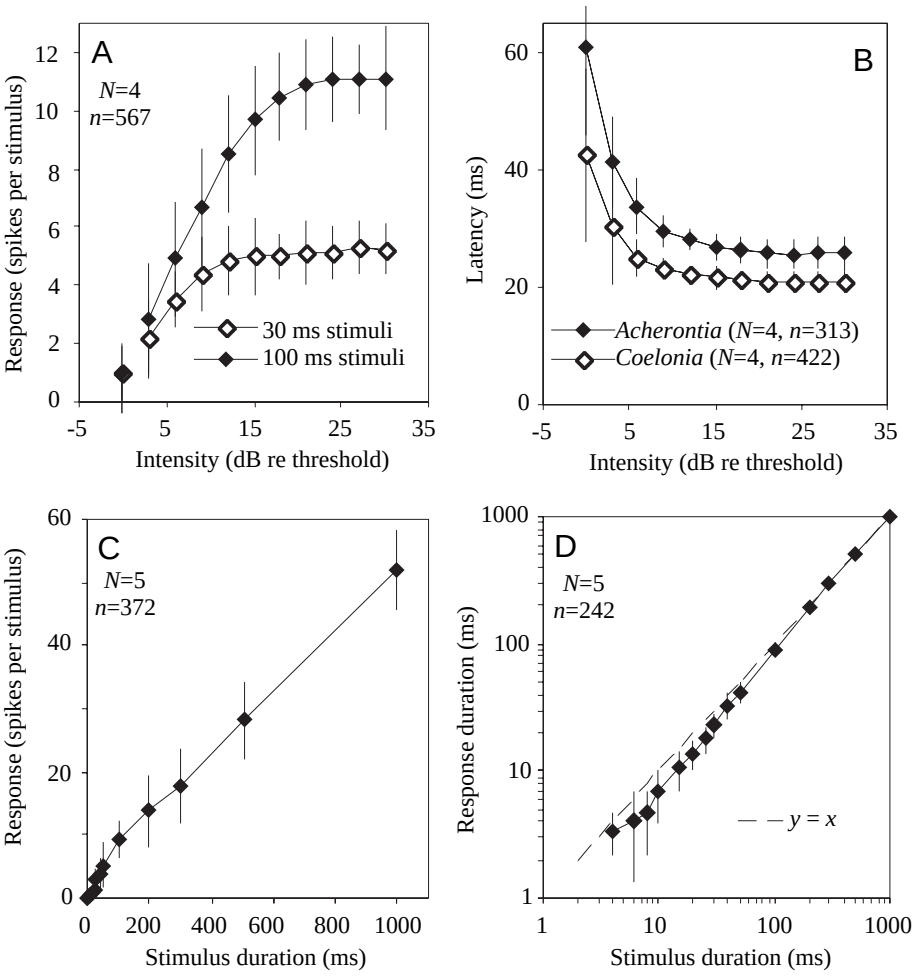

Fig. 8. Characterization of the acoustically elicited activity of the galeal elevator muscle. (A) Response/intensity curves from *Acherontia atropos* showing the increase in the mean number of muscle potentials per stimulus with increasing stimulus intensities above threshold. (B) Plots of the mean latency compared with the stimulus intensity above threshold in *Acherontia atropos* and *Coelonia mauritii*. (C) Change in the mean number of muscle potentials per stimulus with increasing stimulus duration in *Acherontia atropos*. (D) Mean duration of the muscle response in *Acherontia atropos* plotted *versus* the stimulus duration. Values are means ± S.D.; *N* is the number of animals, *n* is the number of stimuli.

the sensitivity, whereas no remaining sensitivity was detectable after subsequent amputation of the ipsilateral pilifer. These results indicate that the elevator muscles in both galeal lobes receive independent auditory input coming only from the pilifer–palp system located on the same side of the head.

Discussion

In hawkmoths, hearing organs sensitive to ultrasonic sounds were hitherto only known from choerocampine species of the subfamily Macroglossinae (=Choerocampinae *sensu* Rothschild and Jordan, 1903) (reviewed by Scoble, 1992). The fact that the hearing organs of these hawkmoths are formed by the mouthparts, the labral pilifers and the labial palps, caused great surprise when they were identified by Roeder et al. (1968, 1970; see also Pye, 1968). Our studies reveal that, despite their different morphology, the same mouthparts form hearing organs in some acherontiine hawkmoth species of the subfamily Sphinginae (=Acherontiinae *sensu* Rothschild and Jordan, 1903).

According to the behavioural data presented here, the hawkmoth subtribe Acherontiina comprises both hearing and (virtually) non-hearing species. Animals of four of the acherontiine species studied responded to acoustic stimulation and, as we will argue below, these responses were elicited by ultrasonic stimuli of biologically relevant sound intensities and frequencies. In contrast, no responses were detected in animals of two other acherontiine species even when they were stimulated with intense sounds. Hearing and non-hearing Acherontiina differ with respect to the morphology of the labral pilifers and the labial palps. The structure of both mouthparts is obviously modified in a similar way in all hearing species studied, indicating that these mouthparts are involved in sound perception. This indication is confirmed by ablation experiments: as in choerocampine hawkmoths (Roeder et al., 1970; Roeder, 1972), the labial palps of Acherontiina serve as accessory auditory structures increasing acoustic sensitivity, whereas the labral pilifers appear to carry the auditory sensory cells. Thus, the depressed palp with its scale-plate and the unilobed pilifer of hearing Acherontiina appear to have similar auditory functions to those of the swollen palp and the bilobed pilifer of Choerocampina. In the following discussion, we will consider the biological significance and the evolution of hearing in hawkmoths in the light of the results reported in this paper.

Behavioural responses, comparisons and functions

Acoustic stimulation at ultrasonic frequencies elicits startle

responses in tethered flying acherontiine and choerocampine hawkmoth species similar to those in many other insects that have been reported to use hearing in the context of bat avoidance (for reviews, see Surlykke, 1988; Hoy, 1992; Hoy and Robert, 1996). In all hearing hawkmoth species studied, the threshold curves of the responses match the ultrasonic frequencies that dominate the echolocation signals of sympatric bats. In *Coelonia solani* and *Xanthopan morgani*, which are both endemic to Madagascar, the best frequencies (60–70 kHz) correspond to the echolocation frequencies of the largest Malagasy insectivorous bat species, *Hipposideros commersoni* (peak frequencies 65–68 kHz, authors' own measurements), whereas in *Acherontia atropos* and *Coelonia mauritii*, which are both widely distributed in the Afrotropics, the best frequencies (20–30 kHz) match the echolocation frequencies of large, fast-flying molossid bats (approximately 15–30 kHz; Fenton and Bell, 1981; and authors' own measurements). Since the tuning of the acoustic responses in large acherontiine species seems to reflect specific adaptations to the echolocation frequencies of some large bat species, hearing in these hawkmoths presumably evolved in response to the predation pressure exerted by these bats.

The thresholds and latencies of the acoustically elicited flight pattern changes in acherontiine and choerocampine hawkmoths are higher than those in noctuid moths (Roeder, 1967), but are comparable with those found in some other hearing insects, for example in mantids and cicindelid beetles (Yager and May, 1990; Yager and Spangler, 1997). However, the acoustic sensitivity of hawkmoth ears is higher than indicated by the flight pattern changes. In *Acherontia atropos*, the minimum thresholds of one of the tongue muscles are approximately 55 dB, and similar thresholds have also been reported for the activity of auditory interneurones in choerocampine hawkmoths (Roeder and Treat, 1970). As in noctuid moths, the flight pattern changes in hawkmoths include changes in flight speed and turning tendencies, whereas cessation of flight activity occurs less often and only in response to intense stimuli (for noctuids, see Roeder, 1967). While the turning tendencies of other hearing moths are typically directed away from the sound source when stimulated at low intensities, the turning tendencies in hawkmoths seem to be non-directional. This absence of directional responses is probably explained by the absence of directional cues provided by the hearing organs: Roeder and Treat (1970) reported that, in choerocampine hawkmoths, no directional information is coded at the level of descending auditory interneurones. In addition to flight pattern changes, the acoustic startle response includes sound production in *Acherontia atropos* and *Xanthopan morgani.* Arctiid moths and cicindelid beetles are known to produce sounds when stimulated acoustically (e.g. Yager and Spangler, 1997). These sounds could be used to startle approaching bats, to signal the distastefulness of the insect and/or to interfere with the bat echolocation system (for a review, see Surlykke, 1988). In hawkmoths, at least in the case of *Acherontia atropos*, the sounds produced contain ultrasonic frequencies (Sales and Pye, 1974). They are therefore likely to be perceptible to insectivorous bats and to be suited for defence against predation by bats. This hypothesis is supported by a single field observation of an *Acherontia atropos* that was attacked by two hunting bats (Mazzucco, 1966); the moth responded by producing a sound that elicited startle manoeuvres by the bats.

In contrast to the other behavioural components of the acoustic startle response of hawkmoths, the tongue movement caused by activity of the galeal elevator muscles is an extremely stereotyped reflex behaviour characterized by a short latency, by low habituation and by the tracking of the stimulus duration. The absence of these movements in a choerocampine species suggests that the tongue reflex is restricted to hearing Acherontiina. The biological significance of the tongue movements remains unclear. Flower-visiting Lepidoptera have been reported to raise the base of the tongue prior to feeding, and these movements have also been attributed to activity of the galeal elevator muscles (Krenn, 1990). In the long-tongued, nectar-feeding acherontiine species (e.g. *Coelonia* species), acoustically elicited tongue raising may initiate tongue retraction

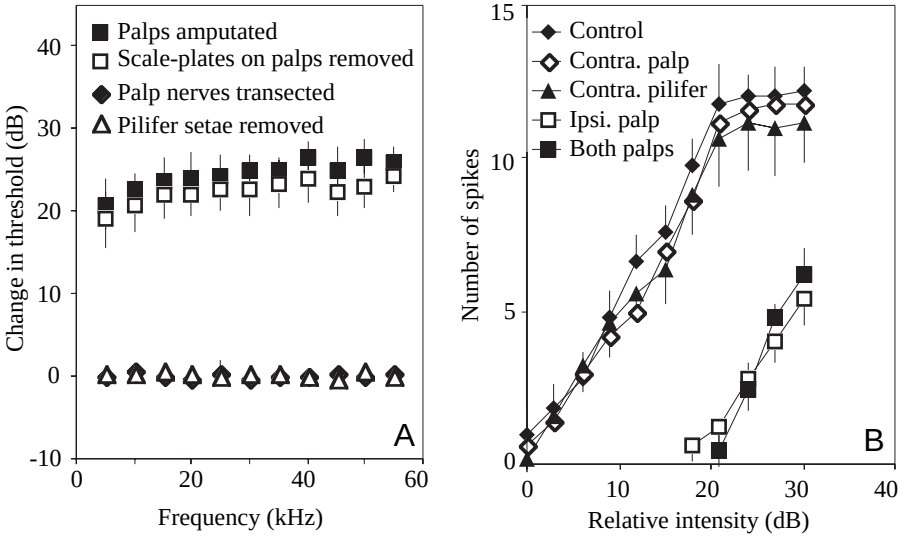

Fig. 9. Changes in galeal elevator muscle activity caused by ablation experiments in *Acherontia atropos*. (A) Mean threshold changes caused subsequent to transection of the labial nerves by removal of the scale-plates on both palps, by amputation of both palps (N=12) and by removal of the sensory setae on both pilifers (N=5). Transection of the nerves and removal of the setae did not affect the sensitivity. (B) Changes in the intensity/response curve caused by deflections of the palps and amputation of the pilifers. Ipsilateral (Ipsi) refers to that pilifer and palp that were on the same side of the head as the galeal lobe we recorded from. Results from a single individual are shown, means and standard deviations are calculated from 10 responses. The frequency used was 25 kHz.

and thus a retreat from the feeding site in the presence of hunting bats. However, this hypothesis does not explain the biological significance of the response in the short-tongued *Acherontia atropos*, which feeds on honey in beehives. The close proximity of the tongue and the hearing organs also suggests that the tongue movement is part of an acoustic feedback loop. Since the tongue is in contact with the pilifers and palps, the movements might cause slight changes in the positions of these mouthparts, thus affecting the auditory input characteristics. However, the occurrence and the significance of such changes remain unclear and must be addressed in further studies.

Evolutionary issues

Although composed of homologous mouthparts, pilifer–palp hearing organs must have evolved independently in acherontiine and choerocampine hawkmoths. It appears to be extremely unlikely that the depressed palp and the scale-plate and the unilobed and slender pilifer found in hearing Acherontiina were derived from the swollen, thin-walled palp and the bilobed pilifer of Choerocampina, or *vice versa*, without affecting the acoustic sensitivity of the system. Instead, the auditory-relevant modifications of these mouthparts presumably evolved independently from the less-specialized condition found in non-hearing hawkmoths. The convergent evolution of pilifer–palp hearing in acherontiine and choerocampine hawkmoths is supported by the distant relationship between these two taxa. Hawkmoths are today divided into two subfamilies, with the Sphinginae including the Acherontiina and the Macroglossinae including the Choerocampina (Rothschild and Jordan, 1903; Hodges, 1971; D'Abrera, 1986; Pittaway, 1993).

Since some of the auditory-relevant modifications of the pilifer–palp system described in the present study have already been noticed by several taxonomists, we are able to make predictions about the distribution of this hearing organ. The deeply depressed palp covered by a scale-plate has been described in five acherontiine hawkmoth genera, including *Acherontia*, *Agrius*, *Coelonia*, *Megacorma* and *Xanthopan* (Griveaud, 1959; Hodges, 1971; D'Abrera, 1986; Pittaway, 1993). These genera comprise approximately 14 species (D'Abrera, 1986).

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