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Morphology and physiology of the prosternal chordotonal organ of the sarcophagid fly *Sarcophaga bullata* (Parker)

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Abstract

The anatomy and the physiology of the prosternal chordotonal organ (pCO) within the prothorax of *Sarcophaga bullata* is analysed. Neuroanatomical studies illustrate that the approximately 35 sensory axons terminate within the median ventral association centre of the different neuromeres of the thoracico-abdominal ganglion. At the single-cell level two classes of receptor cells can be discriminated physiologically and morphologically: receptor cells with dorso-lateral branches in the mesothoracic neuromere are insensitive to frequencies below approximately 1 kHz. Receptor cells without such branches respond most sensitive at lower frequencies. Absolute thresholds vary between 0.2 and 8 m/s^2 for different frequencies. The sensory information is transmitted to the brain via ascending interneurons. Functional analyses reveal a mechanical transmission of forced head rotations and of foreleg vibrations to the attachment site of the pCO. In summed action potential recordings a physiological correlate was found to stimuli with parameters of leg vibrations, rather than to those of head rotation. The data represent a first physiological study of a putative predecessor organ of an insect ear.

Keywords: Vibration; Proprioception; Evolution; Insect

1. Introduction

Chordotonal organs are widespread among insects. Their function is to monitor various kinds of mechanical stimuli, such as proprioceptive, vibrational or acoustic stimuli. Corresponding to the numerous functions, these organs are found in various parts of the insect body. Chordotonal organs consist of one to several hundred units, the scolopidia. Each unit is usually composed of four cells: a scolopale cell, a cap cell, a sensory cell and a sheath cell (Field and Matheson, 1998). In the ventral prothorax of flies a scolopidial sensory organ of unknown function exists, the prosternal chordotonal organ (pCO). This organ is supposed to be the evolutionary precursor organ of the hearing organs found in some parasitoid fly species (Lakes-Harlan and Heller, 1992; Edgecomb et al., 1995; Lakes-Harlan et al., 1999). In general, insect hearing organs were hypothesised to have evolved from chordotonal organs (Yack and Fullard, 1990; Boyan, 1993; van Staaden and Römer, 1998). The pCO of flies was assumed to be involved in head posture control (Hengstenberg, 1991). In accordance with this hypothesis, fibres of pCO receptor cells of *Calliphora erythrocephala* (Calliphoridae) project into motoneuronal neuropiles, which control the neck muscular system (Milde et al., 1987). However, recent investigations do not confirm a role of the pCO in controlling the head position (Gilbert and Bauer, 1998). Instead, a hypothesis has been put forward that the organ is involved in perception of substrate vibrations (Lakes-Harlan et al., 1999). Substrate vibrations are transmitted over the whole body of small insects and might therefore also be registered by sense organs, which are not in direct contact with the substrate (Cocroft et al., 2000). Such vibrational stimuli might cover a large frequency range, such as in the cicada *Okanagana rimosa* where airborne sound induces substrate vibrations up to 10 kHz (Stölting et al., 2002). However, most vibration receptor organs were tested physiologically in a frequency range restricted to

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much lower frequencies (Kalmring et al., 1978; Kühne, 1982; Büschges, 1994; Sauer and Stein, 1999). Whether high-frequency substrate vibrations are used for song recognition or localisation is unknown.

In this paper, we investigate basic anatomical and physiological properties of the pCO, as a putative predecessor organ of an insect ear. We tested the physiological properties of single receptor cells with direct mechanical stimulation up to 10 kHz, but also in the context of a function as vibration receiver. Additionally, we analysed aspects of the neuronal network by recordings of ascending interneurons.

2. Materials and methods

2.1. Preparation

We used specimen of both sexes of *Sarcophaga bullata* var. Ivory (Parker) from a laboratory stock. The species has been renamed *Neobellieria bullata* (Parker) (Shewell, 1989), however, final taxonomic nomenclature seems not to be settled and both names are still in use. Therefore, we will use the still more common name, *Sarcophaga bullata* (Parker). Animals were fixed with wax dorsal side up to a plastic holder. The head was bent upwards and fixed with wax to a thin wire holder. For stabilisation the anterior dorsal thorax was waxed to the fixed head. Thereafter, thoracic cuticle was removed dorsally and the nervous system was exposed (Fig. 1A). The large flight muscles were removed by tissue coagulation to a hot spatula. Care was taken not to damage the nervous system. The central nervous system was kept moist with saline (128 mM NaCl, 10.2 mM KH_2PO_4, 7 mM Na_2HPO_4, 0.4 mM $CaCl_2 \cdot H_2O$ and 0.15 mM $MgCl_2 \cdot 6H_2O$).

2.2. Signal generation and mechanical stimulation

Stimuli were computer-generated sine wave pulses of 50 ms duration with 2 ms rise and decay time in a frequency range from 0.1 to 10 kHz. Each stimulus was presented five times with a repetition rate of two per second. A piezo-element (P-840.01, Physik Instruments) was used for stimulation of the pCO and of the foreleg, respectively (Figs. 1A and B). The vibratory stimuli are characterised by the frequency of the sinusoidal oscillation and by stimulus intensity, which can be expressed either in terms of acceleration, velocity or displacement (Dambach, 1989). The piezo-element has been calibrated with an accelerometer (Bruel & Kjaer 4369; flat frequency response 2 Hz–12 kHz) connected to an amplifier (Nexus 2690). The programs for signal generation and data evaluation were written with the software development kit Lab View 4.01 (National Instruments). Signals were converted with the DAQ-board AT-Mio 16E1 (National Instruments) at 500 kHz sampling rate. The piezo-element was driven via an amplifier (Hewlett-Packard HP-6824A). This experimental setup allowed delivery of stimuli in a frequency range from 100 Hz to 10 kHz, with minimum acceleration values from 0.03 to 5 m/s^2, respectively.

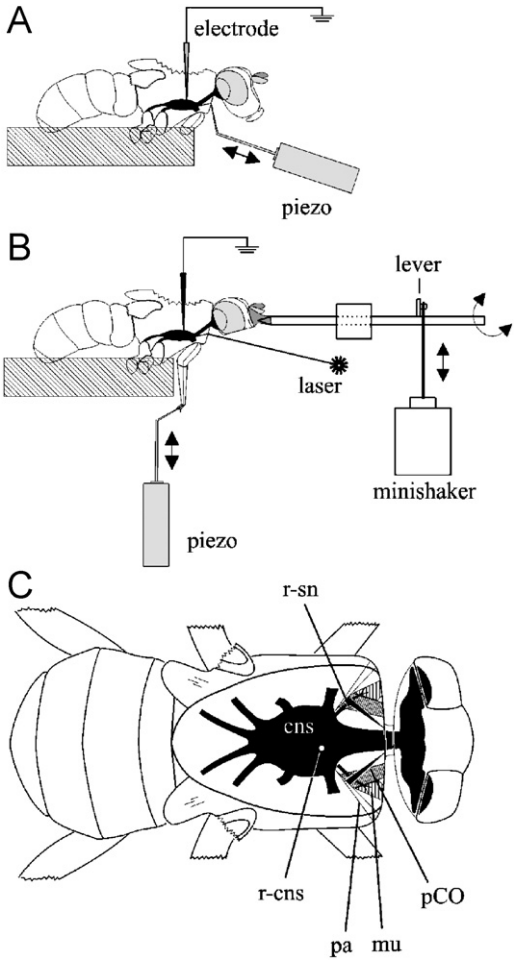

Fig. 1. Experimental setups for investigations of the physiology and function of the prosternal chordotonal organ (pCO) of *Sarcophaga bullata*. (A) Setup for direct stimulation of the pCO with a piezo-element attached to the insertion site of the sensory organ. Potentials were recorded from the frontal nerve or from the CNS. (B) Experimental setup for investigations of the vibrations at the pCO elicited by head rotations and by leg vibrations. Head rotation was achieved by a lever connected to the minishaker; leg vibration by a piezo-element connected to the leg. The stimulus transmission was measured by a laser-interferometer. (C) Schematic dorsal view of a fly depicting the central nervous system (CNS) and recording sites (summed sensory nerve recordings: r-sn; intracellular recordings within the CNS: r-CNS). The sensory organ (pCO) is attached to the prosternal apodeme (pa) and embedded in musculature (mu).

For direct stimulation of the pCO, the piezo-element was connected with wax to the presternum of a fly via a thin needle attached to the vibrating top of the piezo. This preparation was used in all single-cell recordings and in most summed action potential recordings from the sensory nerve of the pCO. Summed action potentials of the pCO were recorded by positioning a sharpened tungsten electrode within or close to the frontal nerve (Fig. 1C, r-sn).

For vibrations of the leg the piezo-element was attached to the foreleg tarsus. The element was arranged approximately in parallel to the tibia and in a 90° angle to the

femur (Fig. 1B). With this experimental setup, summed action potentials were recorded from the pCO (see above) or from the femoral chordotonal organ (feCO). Recordings of the response of the feCO were performed with the tungsten electrode placed within the femur of the foreleg.

2.3. Electrophysiology and data recording

The extracellularly recorded potential was amplified 1000× (custom-built amplifier) and displayed on an oscilloscope and headphones. For intracellular recordings a glass microelectrode (1.0/0.5 outer diameter/ inner diameter; 80–150 MΩ resistance) was inserted into the prothoracic neuromere ipsilaterally between midline and the estimated projection site of the sensory neurons (Fig. 1C, r-CNS). In pilot experiments this area was found to provide the highest probability for successful recordings. All recordings were probably from axonal parts of sensory cells or interneurons, because subthreshold potentials have not been recorded. Single-cell recordings were amplified by an impedance matched SEC-05L amplifier (NPI-Instruments). Neurones were iontophoretically stained with 5% Lucifer-Yellow in 1 M Lithumchloride for 15 s up to 3 min by hyperpolarising currents (0.3–2.5 nA). Neurons were stained in 14 out of 37 preparations.

For all summed action potential recordings the threshold was detected via headphones. The lowest intensity rendering a stimulus correlated nerve activity at a given frequency was defined as threshold. In some experiments the gliding length of the response curve was calculated within a time window from 0 to 100 ms after stimulus onset and compared to a value 100 ms before the stimulus (“spontaneous activity”; Stumpner and Lakes-Harlan, 1996). Calculated thresholds and thresholds obtained with headphones did not differ. In single-cell recordings, curves for frequency intensity characteristics (IC) were calculated by the LabView software. In a spreadsheet program (MS Excel) the dynamic range of the receptor cell response was linearly extrapolated to threshold. For this purpose, spontaneous activity was determined during 0.5 s immediately before each IC measurement and threshold was defined as 0.5 spikes above spontaneous activity. The displayed data points represent means and standard deviation of the responses to five successive stimuli.

2.4. Laser measurements

In search for the natural function of the pCO two experiments with different conditions of stimulation were designed (see below). Firstly, the mechanical vibrations of the pCO attachment site were analysed with a combined laser vibrometer/interferometer (Polytech OFV-2100). The instrument permits non-contact measurements of both the velocity of small-amplitude, high-frequency vibrations and the amplitude of large low-frequency deflections. In order to enhance the quality of the measurements, a small glass sphere (Scotchlite 7610) weighting about 0.2 μg was placed on the attachment site. The signal was evaluated by fast Fourier transformation with a spectrum analyser (Hewlett-Packard 3565; for further details see Meyer and Elsner (1995)).

For applying head rotations a small device was constructed consisting of a metal axis (Fig. 1B) to which the middle of the fly’s head was attached. At the other end of the axis a minishaker (Bruel & Kjaer 4810) was connected to a lever. The lever transferred the linear movements of the minishaker into rotational movements of the axis and the head, respectively. The angle of rotation was varied from $+5^\circ$ to -5° and the minishaker delivered frequencies from 1 to 100 Hz. Care was taken to adjust the axis in parallel to the fly while the tip was pointing directly to the neck so that rotations of the axis did not shift the head to any one side.

For applying leg vibrations, the piezo-element was attached to the foreleg tarsus, with vibrations applied approximately in parallel to the tibia. Frequencies from 100 Hz to 10 kHz were tested with a constant amplitude of 0.8 μm and the amplitude of the vibrations of the pCO attachment site was measurement.

2.5. Histology

The nervous system was fixed for 30–60 min in 4% paraformaldehyde and dehydrated in an ethanol series. After clearing in methylsalicylate the neurones were viewed in wholemount with a fluorescence microscope (Leica, Dialux 20) and was documented by photographs. Additionally, a wholemount drawing of the neuron was made using a drawing tube. Thereafter the nervous system was embedded in polyester wax and horizontally or transversally sectioned on a microtome (Reichert-Jung, 1130 Biocut, 10 μm).

Backfills of the sensory neurones of the pCO were made with 5% Neurobiotin in 1 M potassium acetate (Stumpner, 1996) filled in the tip of a glass capillary. The pCO was cut close to the sensory nerve and carefully placed into the capillary. Using Neurobiotin it was possible to completely stain sensory axons within approximately 15 min. The Neurobiotin marker was visualised by standard techniques with a biotin–avidin system (Vector ABC kit, (Stumpner, 1996)).

The anatomy of the ventral prothorax was revealed after fixation of the fly in 4% paraformaldehyde for at least 2 h and dehydration in a rising ethanol series. Specimens were embedded in Agar 100 (Agar Scientific) via methylsalicylate and sectioned (2 μm) on a microtome (Leica RM 2165). Sections were counterstained with Methylene blue. The neuroanatomy of the thoracico-abdominal ganglion was revealed in transverse section series (10 μm) after fixation in 4% paraformaldehyde and embedding in polyester wax. Structures were made visible in haematoxylin staining: Sections were stored in 3–5% Fe^{III+}-solution for 5 min and in 1% haematin solution for another 5 min. Washing in water results in a blue staining with high visual

contrast. Finally, the sections were dehydrated and embedded in Roti Histokit (Roth).

3. Results

3.1. Anatomy of the pCO of S. bullata

Sclerotised and membraneous skeletal elements can be found at the ventral prothorax (Fig. 2A). The presternum (ps) is a rigid cuticular structure at the midline. Dorsally the presternum is in contact with the laterocervical sclerites (lc) to which neck muscles are attached. These sclerites are involved in movements of the head. Ventrally the presternum has contact to the probasisternum (pbs). Laterally the large folded membranes (psm) span from the laterocervical sclerites (lc) to the coxae (co) and to the presternum.

The paired scolopidial organ extends from the prosternal apodeme (ap) to the lateral edge of the presternum (Fig. 2B). The long axis of the sensory organ extends in an almost horizontal plane in close proximity to prothoracic muscles. Each sensory organ is a scolopidial organ consisting of approximately 35 homogeneously sized scolopidia (Fig. 2C). The scolopales are approximately 3 μm in diameter and are located within one plane of the sensory organ. Elongated cap cells (cc) extend to the attachment site. The axons of the receptor cells form a sensory nerve joining after 200–300 μm the frontal nerve, which enters the thoracico-abdominal ganglion. The axons form a dense bundle within the central nervous system (CNS) and run through the dorso-lateral tract of the ventral fasciculus (DLV) to each thoracic neuromere (Fig. 3A). In each thoracic neuromere collaterals branch off the axon bundle. Histologically each thoracic neuromere has a dense neuropile, the medial ventral association centre (mVAC; Figs. 2D and E) situated close to the DLV and the ventro-medial tract of the ventral fasciculus (VTV). Many median projecting branches of the sensory axons

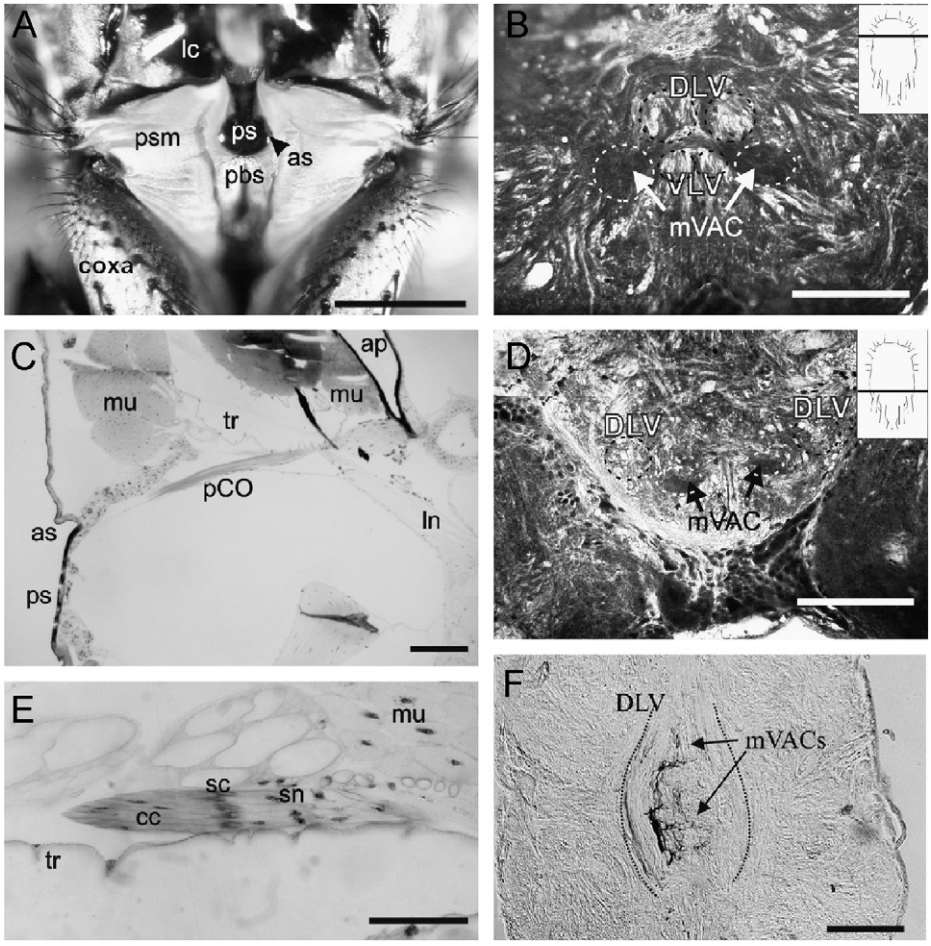

Fig. 2. Photomicrographs of the sensory system and the central nervous system. (A) Frontal view of the prothorax of *S. bullata*. The arrowhead points towards the attachment site of one prosternal chordotonal organ (as). lc: lateral cervicale, pbs: probasisternum, ps: presternum, psm: prosternal membrane. Scale: 500 μm. (B) Horizontal section of the prothorax showing the position of the prosternal chordotonal organ (pCO). ap: apodeme, ln: legnerve, mu: muscle, tr: trachea. Scale: 100 μm. (C) Longitudinal section of the prosternal chordotonal organ. cc: cap cells, sc: scolopidia, sn: sensory neuron. Scale: 50 μm. (D, E) Transverse sections of the thoracico-abdominal ganglion complex at two different positions indicated on the right. DLV: dorsolateral tract of the ventral fasciculus, VTV: ventromedian tract of the ventral fasciculus, mVAC: median ventral association centre. (F) Horizontal section of the thoracico-abdominal ganglion with labelled sensory fibers. The DLV is outlined and the mVAC of the pro- and mesothoracic neuromere is indicated. Scales D–F: 100 μm.

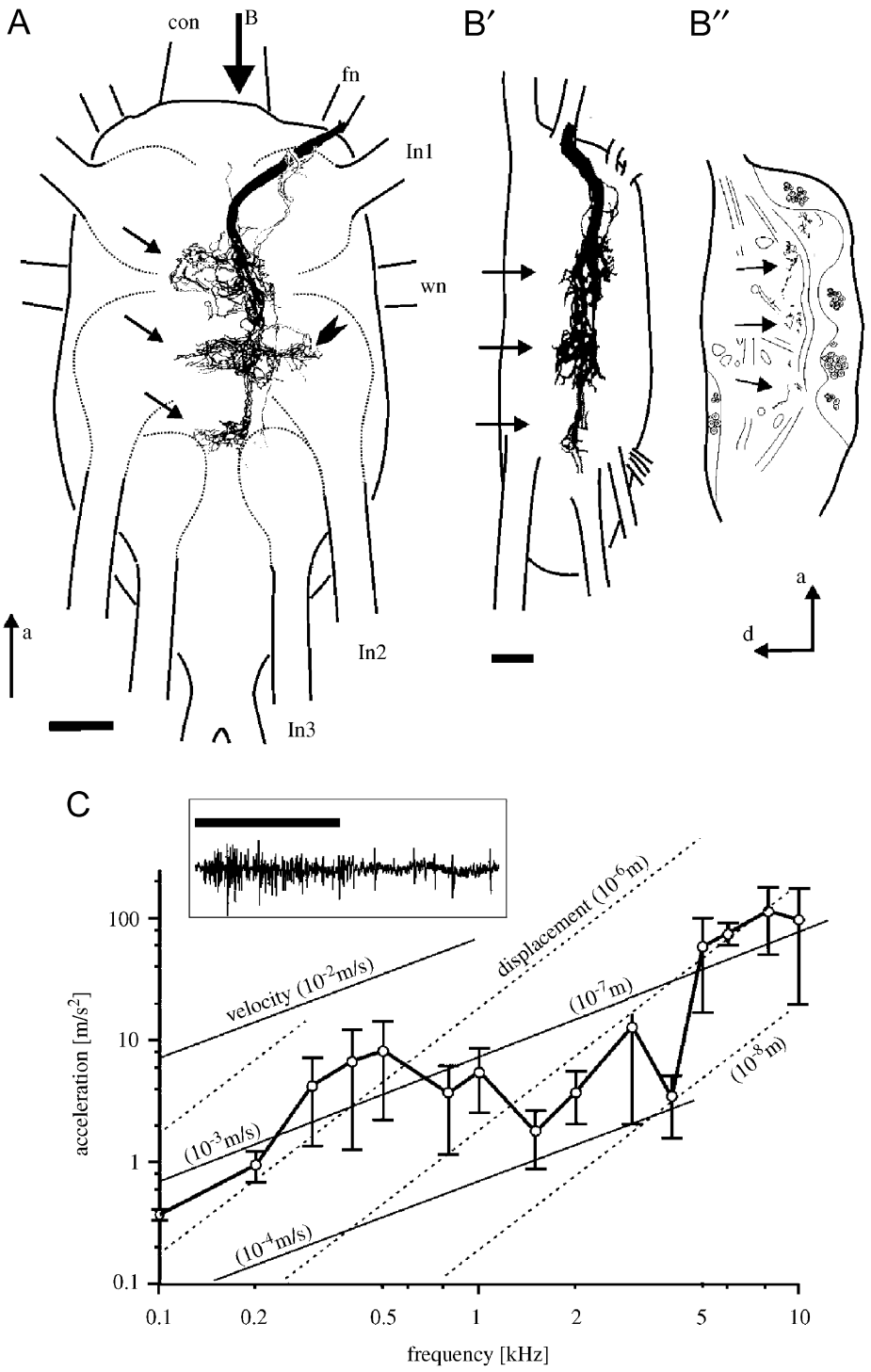

Fig. 3. (A) Central projections of the sensory fibers of the pCO in the thoracico-abdominal ganglion. The sensory axons enter the CNS via the frontal nerve (fn) and have collateral branches in all three thoracic neuromeres (arrows). The dorso-lateral branches in the motoneuropile of the mesothoracic neuromere are indicated by an arrowhead. (B) Lateral view (B′) and parasagittal section (B″) showing the main axonal tracts and the arborisation in the median ventral association centre (arrows). The level of section is indicated in A. (C) Threshold of acceleration of the pCO (mean with SD; recorded extracellular at the frontal nerve; see inset for an example of the recording). The iso-velocity and iso-displacement lines are also given. a: anterior; con: neck connective; d: dorsal; ln1-3: leg nerve 1–3; wn: wing nerve. Scale bars: 100 μm.

terminate in the mVAC (Figs. 2F and 3B). Several fibres cross the midline of the thoracico-abdominal ganglion and project to contralateral areas. Additionally, receptor fibres were also found in a dorso-lateral motor neuropile within the mesothoracic neuromere (arrowhead in Fig. 3A).

3.2. Biophysical evaluation of the natural stimulus

In order to evaluate the range of mechanical stimuli that might act on the pCO two experiments were designed. Firstly, we tested whether the pCO might be involved in

measuring head movements in an experiment in which rotational movements were applied to the head. The resulting movements of the attachment site of the pCO were measured with a laser-interferometer (see Fig. 1B). Head rotations from 1 to 30 Hz elicited phase-coupled movements of the presternum (Fig. 4A). The phase coupling disappeared at higher frequencies presumably due to resonant properties of the fly's skeletal system. The applied rotational movements ($+5^\circ$ to -5°) resulted in relatively large amplitude displacements (up to 10 μm) at the presternum.

Secondly, we tested whether substrate vibrations are transmitted via a foreleg to the presternite. Experimental vibratory stimulation of the foreleg with frequencies between 100 Hz and 10 kHz with an amplitude of 0.8 μm resulted in a phase coupled vibration of the presternum (Fig. 4B). The displacement amplitudes of the vibrations were frequency dependent and ranged from 0.002 to 0.1 μm (compare spectral data in Lakes-Harlan et al., 1999).

Thus, the presternum vibrates in response to both stimuli. In following electrophysiological experiments we simulated the hypothetical natural stimulus and applied low frequency–high amplitude stimuli (representing head rotation) and high frequency–low amplitude stimuli (representing leg vibration) directly to the pCO attachment site. With low frequency–high amplitude stimuli in the frequency range from 0.1 to 50 Hz and displacements up to 50 μm no stimulus correlated activity was found in summed action potential recordings (data not shown). By contrast, a response was obvious in experiments with high frequency–low amplitude stimuli.

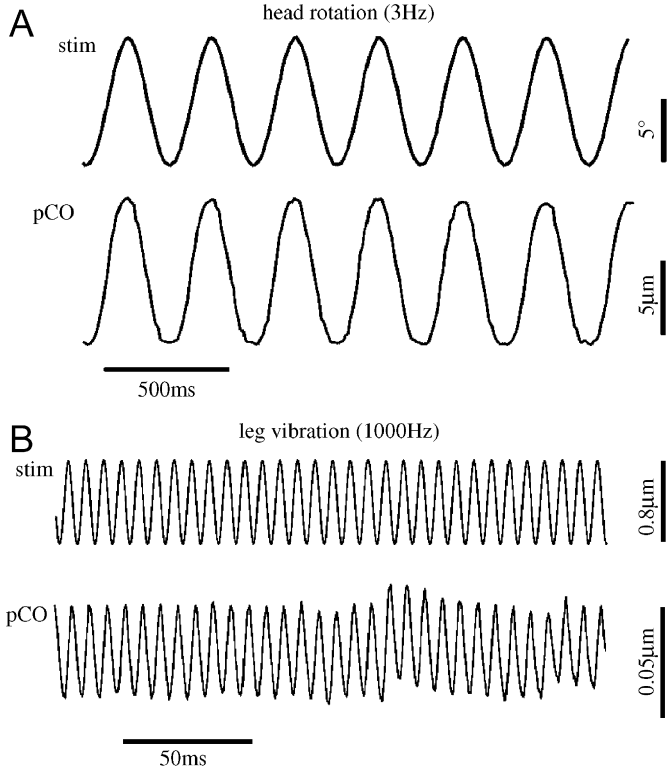

Fig. 4. Biophysical measurements of the stimulus transmission to the pCO. (A) A rotational stimulus applied to the head (stim) and the resulting movement of the attachment site of the pCO. (B) A vibratory stimulus applied to the foreleg (stim) and the resulting movement of the attachment site of the pCO.

3.3. Receptor cells

Extracellular recordings from the sensory nerve showed a varying threshold of acceleration in the frequency range from 100 Hz to 10 kHz (Fig. 3C). Lowest thresholds were seen below 200 Hz and further minima occurred at 1.5 and 4 kHz, with a large interindividual variability. The curve partially parallels iso-displacement lines (with a minimum of about 10^{-8} m) and iso-velocity lines (with a minimum of about 10^{-4} m/s; Fig. 3C).

In single-cell recordings, two types of receptor cells could be identified by both, morphology and physiology. Type 1 receptor cells ($N = 3$) responded in the whole tested frequency range (Fig. 5B; not tested below 200 Hz) and type 2 receptor cells ($N = 3$) did not show any responses to frequencies below 1 kHz (Fig. 5B). Both cell types had distinct minimal thresholds at 1.5 kHz and both cell types were found with successive recordings in one preparation. Both cell types have terminations of almost equal density in all thoracic neuromeres (Fig. 5C). However, type 1 has no dorso-lateral branch, whereas type 2 cells possess branches into the dorso-lateral motoneuropile (Fig. 5C).

A variety of intensity response curves were found in the pCO independent of the receptor cell type. At different frequencies, each cell had consistent response curves. The different kinds of response curves are especially obvious when compared for the same frequency (e.g., 1.5 kHz; Figs. 5D–F). Some cells had bell-shaped intensity response curves (Fig. 5D). These cells typically were not spontaneously active. Other cells showed steadily increasing responses with increasing acceleration (within the tested range; Fig. 5E). Thirdly, cells were found, which were only weakly activated by the stimuli with response curves showing almost no dependence on intensity and frequency (Fig. 5F). Again, all response types were also found in successive recordings in one preparation.

The discharge pattern of most cells was phasic-tonic (Fig. 5A). The pattern depended on intensity as well as on frequency. For example, one cell had a largely tonic discharge at 10 m/s^2, which changed to a mainly phasic discharge pattern at 50 m/s^2 (Fig. 5G). Interestingly, other cells had prominent off-responses (e.g. at 100 Hz, 0.8 m/s^2; Fig. 5H). The discharge pattern of this cell depended not only on stimulus intensity but also on stimulus frequency. With increasing frequency this cell changed its responses to a more phasic-tonic pattern. Shortest latencies of the tested cells varied between 2 and 4 ms.

3.4. Interneurones

Recording and staining of ascending interneurons that transmit vibratory information from the pCO to the brain

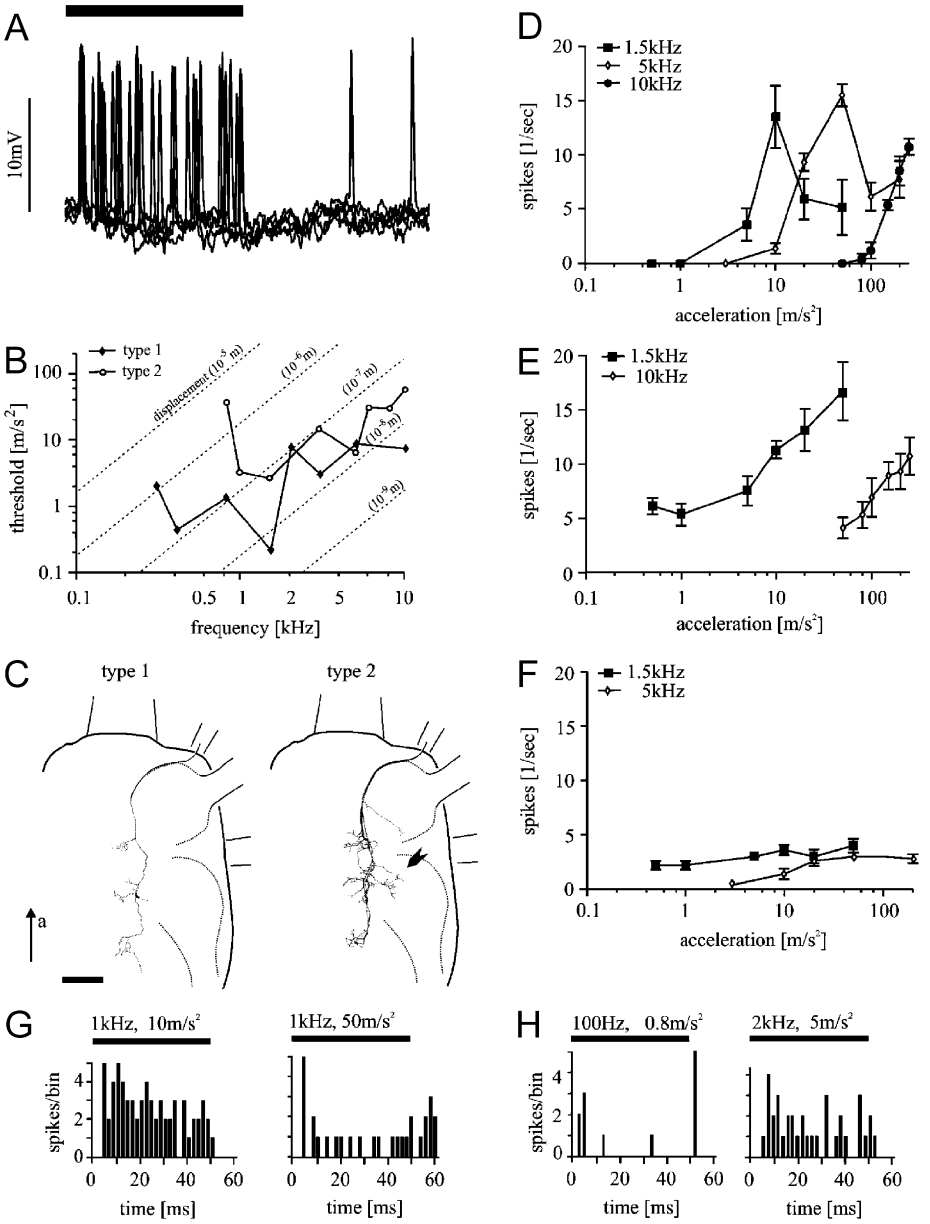

Fig. 5. Physiology of single receptor cells of the pCO. (A) Overlay of five recordings showing the sharp onset of the response with 2 ms latency (stimulus: 50 ms duration, 2 kHz frequency, 5 m/s^2 acceleration). (B) Acceleration threshold curves of a types 1 and 2 cells in comparison with iso-displacement lines. (C) Drawing of a type 1 sensory fiber and a type 2 sensory fibers in a preparation with three stained cells. The arrowhead marks the dorso-lateral branches. (D–F) Intensity response curves showing the different kinds of responses characteristics: (D) type 2 cell; (E, F): type 1 cells; mean with SD). (G, H) Peri-stimulus-time histograms of neuronal responses to five consecutive stimuli. Stimulus duration is indicated by the bar above the histogram: (G): type 2 cell; (H) type 1 cell.

was performed primarily with the purpose to identify putative homologues of auditory interneurons in hearing flies. Various interneurons with the same basic physiology as the receptor cells were found and two types were also morphologically identified. The first type (IN_1) is a contralaterally ascending interneuron with dense presumably dendritic arborisations in the pro- and mesothoracic neuromere (Fig. 6A). The soma lies at the ventral border between the meso- and metathoracic neuromeres. The main neurite crosses the midline in the mesothoracic neuromere and presumably dendritic branches (fibers with smooth structure) extend into both halves of the pro- and mesothoracic neuromeres. The dendrites overlap with the termination area of receptor cell axons of the pCO. The IN_1 was not spontaneously active and stimulation of the pCO elicited a weak phasic response (Figs. 6B and C). The second type of identified interneuron (IN_2) has an ipsilaterally ascending axon and its soma is located in the medial part of the metathoracic neuromere. The presumed dendrites branch in each thoracic neuromere with crossing fibers in the pro- and mesothoracic neuromere. The branching area overlaps with those of the sensory axons

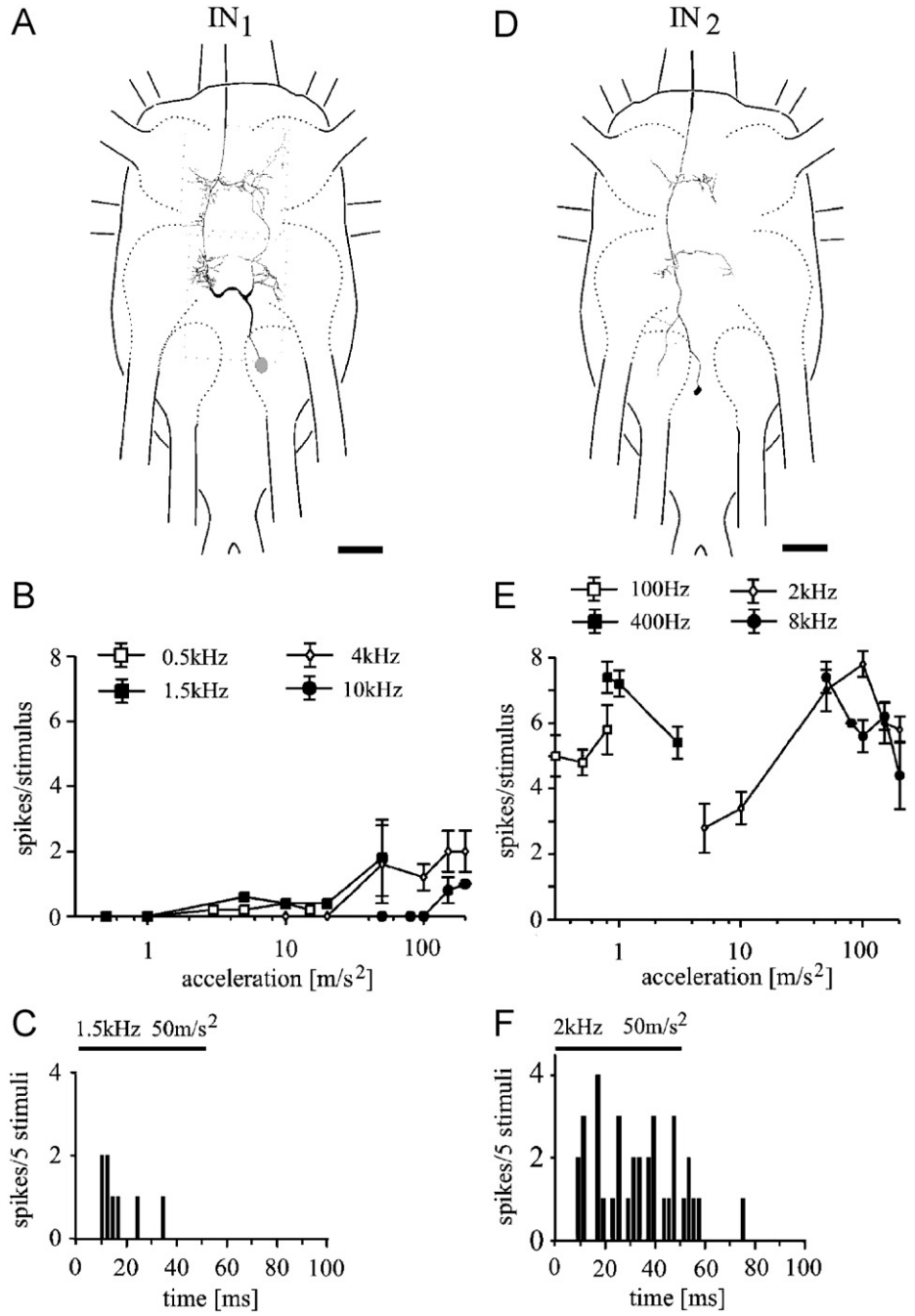

Fig. 6. Morphology and physiology of two types of ascending interneurons (IN_1, IN_2) responding to stimulation of the pCO. (A, D) Drawing of the wholemount morphology of the neurons (the soma position in A is reconstructed from serial sections with immunocytochemical amplification of the Lucifer Yellow signal). (B, E) Intensity response curves for different stimulus frequencies (mean with SD). (C, F) Peri-stimulus-time histograms of neuronal responses to five consecutive stimuli. Stimulus duration is indicated by the bar above the histogram.

of the pCO. The IN_2 responded phasic-tonically with bell-shaped intensity response curves when the pCO was directly stimulated (Figs. 6E and F). The shortest latencies of interneurones ranged from 5.5 to 8 ms.

3.5. Function of the pCO

The experiments with direct stimulation of the pCO showed that receptor cells responded in the high-frequency range. Therefore, we investigated whether vibrations of the leg activated the receptor cells. The pCO responds to vibrations of the leg with frequency dependent thresholds in the 10–100 m/s^2 range (Fig. 7). No response could be elicited with stimuli below 500 Hz. Although this experiment demonstrates that the pCO can be excited by high-frequency substrate vibrations, it appears to be rather insensitive. By comparison, the feCO is 2–3 orders of magnitude more sensitive to substrate vibrations (Fig. 7).

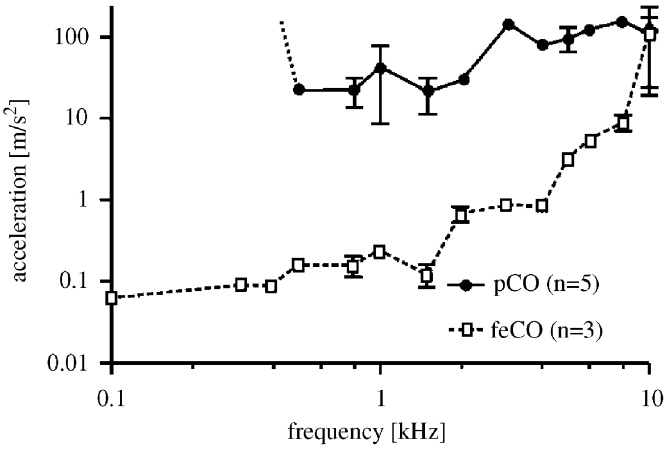

Fig. 7. Comparison of the threshold curves of two sensory organs to foreleg vibrations (mean with SD). The sensory responses have been recorded extracellularily from the frontal nerve (prosternal chordotonal organ; pCO) and from the leg nerve (femoral chordotonal organ; feCO).

It reacts to stimuli below 500 Hz and has a frequency-dependent threshold with a best sensitivity in the frequency range 100 Hz–1.5kHz with approximately 0.1 m/s^2.

4. Discussion

4.1. Function of the prosternal chordotonal organ (pCO)

Two main hypotheses have been put forward for the function of the pCO. Firstly, it was proposed to monitor head movements, as do other sense organs close to the neck (Hengstenberg, 1991; Gilbert and Bauer, 1998). Our results show that the sclerite to which the sense organ is attached is mechanically phase-coupled to head rotations up to 30 Hz. In this experiment only one type of movement was tested. Head movements are much more complex and further functional studies are required to fully evaluate the role of the sensory organs. Nevertheless, our experiment already shows that the sternite moves when the head is rotated at low frequencies and it is likely that natural head movements are rather slow movements. Electrophysiological experiments, in which such low frequency stimuli of high amplitude were applied to the pCO, however, did not reveal stimulus correlated activity. Although it might be possible, that single cell responses were not detected in the summed nerve recordings, the results are in agreement with lesion experiments. Removal of the pCO did not change head control in *S. bullata* (Gilbert and Bauer, 1998). Thus, even if we have not applied all stimuli potentially occurring during head movement, monitoring head movements seems an unlikely function of the pCO.

Secondly, it was hypothesised that the pCO could monitor substrate vibrations transmitted via the legs (Lakes-Harlan et al., 1999). Our laser measurements show that substrate vibrations are transmitted to the sense organ. Substrate vibrations are probably transmitted across the whole body, where they might be used for detection of the stimulus direction, similar to the situation in treehoppers (Cocroft et al., 2000). Our data demonstrate that the pCO responds to vibrations in the frequency range up to at least 10 kHz. With increasing frequency the overall threshold of acceleration is raised, up to rather high values. Single cells had thresholds below those found in summed nerve recordings (e.g. at 1.5 kHz, 0.2 m/s^2 compared to 2 m/s^2). Such values are similar to thresholds of other chordotonal cells in different species. Receptors with thresholds of approximately 0.1–1 m/s^2 have been recorded in the prothoracic feCO of locusts (Kühne, 1982). Specialised vibration receivers such as the subgenual organ of bushcrickets and cockroaches, however, are much more sensitive to vibrations. Typical values for the threshold of acceleration of the subgenual organ of *Periplaneta* sp. are 2×10^{-4} m/s^2 at 1.4 kHz and below 1 m/s^2 at 8 kHz (Autrum and Schneider, 1948). In *Decticus verrucivorus* (Tettigoniidae) receptor units of the complex tibial organ were found responding to vibrations of $1–5 \times 10^{-3}$ m/s^2 at 1 kHz (Kalmring et al., 1994). Thus, sensory organs known as proprioceptors such as the feCO appear to be one to two magnitudes less sensitive than specialised vibration receivers like the subgenual organ. Flies do not have a subgenual organ (Lakes and Pollack, 1990), thus they have to register vibrations with other sense organs. The major chordotonal organ in the fly leg, the feCO, responds more sensitive to vibrations than the pCO. Nevertheless, it might be important that the pCO can detect substrate vibrations despite the insensitivity, because the information of both organs might converge in the CNS, e.g., for intensity discrimination. The relative insensitive threshold leaves room for a further hypothesis on the function: the pCO might also be involved in monitoring vibrations during flight.

Our direct stimulation of the sensory cells allowed a function-independent evaluation of basic physiological parameters of a scolopidial organ. In the pCO of the fly at least two physiologically different receptor cell types occur. They do not differ in their temporal response pattern (phasic-tonic), but in the frequency range. One type responds to frequencies below 1 kHz while the other is very insensitive in this range. This can be interpreted as a range fractionation of the frequency–intensity domain to increase the dynamic range of the entire sensory organ. Similar properties have been suggested for the feCO of locusts (Matheson, 1992). On the other hand, the different physiological properties correlate with morphological differences (e.g. type 2 receptor cells have lateral branches in the mesothoracic motoneuropil) and this suggests different tasks of the two types of receptor cells and therefore differences in their neuronal connections.

4.2. Evolutionary aspects

In principal, scolopidial receptor cells measure amplitude, velocity and/or acceleration of a mechanical stimulus such as vibration or sound. In combination with

anatomical data of the location of the chordotonal organs and the axonal projections this has led to the hypothesis that insect auditory organs evolved from proprioceptors or vibration receptor organs (Boyan, 1993; Fullard and Yack, 1993; van Staaden and Römer, 1998). For the Diptera it has been proposed that the pCO is the evolutionary precursor organ of the ear, found in some fly species (Lakes-Harlan and Heller, 1992; Robert et al., 1992; Lakes-Harlan et al., 1999). This hypothesis has been supported by anatomical data of the position and central projection of the sensory organ. The hypothesis is now further strengthened by the physiological characteristics of the sense organ.

We have demonstrated that the pCO is sensitive to vibrations in a large frequency range. Thus, the receptor cells are able to transduce stimuli well above the frequencies seen in proprioception. This characteristic might be important for the evolutionary transition to auditory receptor cells. Furthermore, a predominantly tonic response pattern is typical for auditory receptor cells of different insects (Rheinlaender, 1975; Römer, 1976; Oldfield, 1984; Kalmring et al., 1996), including Diptera (Stölting et al., unpublished results), although in ormiine flies (Tachinidae) strictly phasic responses have been found as well (Mason et al., 2001; Oshinsky and Hoy, 2002). The receptor cells of the pCO of *S. bullata* respond phasic-tonically to sinusoidal stimulation, whereby the tonic component is prevailing in certain frequency and intensity ranges. With respect to evolution, this would indicate that the predecessor species of a hearing fly species had a sense organ sensitive to substrate vibrations. Since the hearing flies use their ears to find hosts for larvae deposition, an additional sensitivity to substrate vibration would be especially advantageous if the host does not only emit sound but also substrate vibrations during signalling. Recent measurements showed that high-frequency vibrations can be recorded from the substrate close to a singing cicada (Stölting et al., 2002).

In addition to the physiology, the morphology of the pCO indicates that this organ can be an evolutionary precursor organ of the dipteran ear. Firstly, all receptor cell axons terminate within all thoracic neuromeres within the thoracico-abdominal ganglion, similar to auditory receptors in hearing flies (Stumpner and Lakes-Harlan, 1996). Most of the branches were found within the mVAC typically being the projection area of auditory organs in insects (Boyan, 1993). Additionally, the anatomy of the pCO terminations resembles that of the anterior chordotonal organ (aCO) of the locust (Pflüger and Field, 1999). The aCO is thought to monitor internal pressure changes and, interestingly, it is also sound sensitive.

Further indications for the evolutionary homology of the hearing and the chordotonal system in flies come from interneurons. Both neurones described in this paper have segmentally repeated dendritic branches which are already known from acoustic interneurons of the tachinid fly *Therobia leonidei* (Stumpner and Lakes-Harlan, 1996). Morphologically very similar neurones to IN_1 are also found in the hearing sarcophagid *Emblemasoma auditrix* (Stölting et al., unpublished results). The IN_2 represents an unusual interneuron of insects, because it has an axon ascending ipsilaterally to the soma. It remains to be shown whether such neurons can also be founding hearing Diptera and which implementations this morphology has for the information processing. In general, the diversity of sensory interneurons in auditory-vibratory systems is large (Römer and Marquart, 1984; Boyan and Fullard, 1986; Boyan and Miller, 1991; Stumpner and Ronacher, 1991; Stumpner and Lakes-Harlan, 1996; Prier and Boyan, 2000; Stumpner and Molina, 2006) and the potential function of single neurones has to be studied in a larger sample than that presented here. It is clear from the data presented here, however, that interneurons involved in processing information from the pCO show a diversity of physiological responses, which fits well into the spectrum of properties encountered in interneurons of related systems.

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