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DETECTION OF AIRBORNE SOUND BY A COCKROACH 'VIBRATION DETECTOR': A POSSIBLE MISSING LINK IN INSECT AUDITORY EVOLUTION

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Summary

Extracellular recordings from nerve 5 in metathoracic legs of *Periplaneta americana* disclose a sense organ that is extremely responsive to vibration but also detects sound (best response near 1.8 kHz) with a sensitivity similar to some insect auditory organs. The energy required from an auditory signal for a criterion response is similar or even smaller than for an optimal vibratory input. Responses originate in the subgenual organs (SGO) in the proximal tibiae, and cross-modal adaptation indicates that the same cells respond to both vibration and sound. Sound is picked up directly on some internal structure, not *via* sound-induced substratum vibration. Adaptation at different frequencies discloses no frequency-selectivity in the SGO. The nerve response is a burst of synchronized impulses at a frequency, f_R, of approximately 300 Hz, that is practically invariant both with sound intensity and within the burst, suggesting that f_R might represent some underlying resonance phenomenon, either of the SGO or of air in the tracheal system. The latter possibility is ruled out by observations made while the tracheae are perfused with $He–O_2$. Similar responses can be recorded from the pro- and mesothoracic legs. Although *Periplaneta* is thought to be deaf and appears to ignore loud tones presented to the home colony, a more sensitive assay detects small leg movements in response to sound, confirming the presence of a functional auditory sense. The SGO is suspended from an expansion of the leg trachea, which may function to enhance sensitivity to vibration. This linkage preadapts the SGO to detect airborne sound transmitted in the tracheal system, and contact vibration may also stimulate the system in part by deforming the tracheae. It is proposed that auditory organs of crickets evolved from an ancestral SGO that already possessed dual responsiveness by subsequently developing effective vibration-isolating filters.

Introduction

The auditory apparatus of crickets (Ensifera), carried in the tibiae of the two forelegs, has been the object of intensive study on several fronts (Huber *et al.* 1989). Each ear is elaborated in the tibia as an array of chordotonal sensilla attached to the wall of the leg's

Key words: sound receptor, vibration detector, subgenual organ, insect ear, auditory evolution, cockroach legs, chordotonal sensilla, *Periplaneta americana.*

main trachea and is closely associated with up to two tympana. In well-studied species, it has one of the most complex acoustic pathways known, with at least four acoustically interacting sound inputs originating at both sides of the body (Larsen *et al.* 1989). As with most aspects of nervous organization (Arbus *et al.* 1991), the means for sound detection presumably arose through evolutionary modification of some other pre-existing system, but this antecedent condition is poorly understood. The cricket auditory organ probably evolved from the adjacent subgenual organ (SGO), present in all six legs (Meier and Reichert, 1990). In bushcrickets (Tettigoniidae), Autrum (1941) established that the auditory organ responds to high-frequency sounds and suggested that the subgenual organ is a detector for contact vibration. Although the tettigoniid SGO also responded to lower frequencies of airborne sound, Autrum (1941) considered that this might be an indirect effect, for instance caused by sound-induced substratum vibration combined with the exquisite vibration-sensitivity of the SGO (Autrum and Schneider, 1948), an idea that has persisted in the literature ever since. How a substratum vibration sensor might transform into a purely auditory organ is not immediately clear.

Cockroaches (Blattariae) are one of the least modified of the older pterygote groups, emerging as a distinct entity in the upper Carboniferous (Hennig, 1981). Along with mantids, termites and a few smaller groups, they share a common ancestry with crickets from Hennig's Orthopteromorpha in the early Carboniferous. Mantids can also detect sound, but use a central metathoracic ear that is not homologous with the organ in crickets' legs (Yager and Hoy, 1986), whereas cockroaches are usually regarded as silent and deaf (Huber *et al.* 1990). The SGO of the cockroach *Periplaneta americana* is the most sensitive known insect vibration detector (Autrum and Schneider, 1948). Features of its neuroanatomy have been described (Schnorbus, 1971; Moran and Rowley, 1975), but its physiology is hardly known at all.

In exploring the mechanoreceptive properties of the metathoracic legs of *Periplaneta*, it became apparent that its SGO can respond to airborne sound as well as to contact vibration, and the present account uses simple recording methods in a first attempt to characterize the organ. The results show that the leg contains a hitherto undescribed sensitive auditory organ which has some unusual properties but detects sound directly. Behavioural evidence suggests that *Periplaneta* can respond to sound, complementing the recent discovery of sound-sensitive interneurones in the same species (Ritzmann *et al.* 1991). It is suggested that the SGO may have arisen as a vibration-detecting system that underwent later modification for enhanced sensitivity through its coupling to an expansion of the air-filled trachea of the leg, analogous to the auditory organs and swimbladder of some fish, and that access to this air pathway provided the crucial modification that pre-adapted the organ to detect airborne sound. The cockroach SGO thus provides a conceptual bridge for understanding the evolutionary transition from a pure vibration detector to a pure sound receptor that, moreover, may have been the actual path of evolution, and refocuses attention on how the auditory organs of crickets came to lose sensitivity to vibration. Finally, the SGO of the cockroach may also illuminate the origin of the tympana of crickets' ears. Although the cockroach leg has an unspecialized surface with nothing resembling a tympanum, we have demonstrated that some of the sound reaches the SGO through the leg's surface and not just *via* the spiracles and

tracheal pathways (Shaw *et al.* 1992; S. R. Shaw, K. M. Kokic and D. S. MacIntosh, in preparation). A brief account of some of the findings has appeared (Shaw, 1990*a*).

Materials and methods

Adult cockroaches (*Periplaneta americana* L.) of both sexes came from a laboratory colony maintained at 21–23 ˚C on a 13 h:11 h light:dark cycle and fed water, rat chow and lunch leftovers. Animals of known age were used 1–3 days following final moult, after the cuticle had hardened; otherwise, lighter-coloured specimens that were also only a few days old were selected. Individual legs (usually metathoracic) were removed from the animal by cutting through the base of the coxa with scissors after brief CO_2 anaesthesia, and were placed for most experiments on a raised platform with a pliable wax surface (Tackiwax, Boekel Industries, Philadelphia, PA). The tibia was left hanging free at the platform's edge in many cases, or was immobilized by surrounding it with insect pins and spots of Tackiwax in cases where mechanical stimuli were to be applied. The method of mounting had no significant effect upon any of the auditory responses described. The platform and associated micromanipulators were mounted on a heavy steel plate supported by a concrete slab on a solid table, using antivibration insulation.

In most experiments, two electrolytically sharpened tungsten electrodes were positioned through the cuticle with micromanipulators to span the coxa–femur articulation or at other locations, or 75 μm diameter insulated copper wires were implanted in the leg, and sealed in lightly with Tackiwax. A ground electrode was inserted in the coxa. Sensory action potentials appeared to be predominantly biphasic with the leading phase negative: this is expected for sensory impulses conducting proximally up the leg to meet two separated recording electrodes, the first (distal) connected to the non-inverting input and the second (more proximal) connected to the inverting input of the differential high-gain preamplifier. Evoked responses were monitored conventionally with analogue and digital storage oscilloscopes, usually signal-averaged and recorded with a plotter or tape recorder. Distal-negative is plotted throughout as a downwards deflection.

Tactile spines on the metathoracic legs occur in five rows, the nomenclature for which is adopted from Chapman (1965) and Chapman and Pankhurst (1967). Spines could be deflected effectively using a precisely timed, brief air puff delivered *via* an electrically operated solenoid valve through a broken-tipped micropipette (Shaw, 1990*b*). Otherwise, mechanical stimuli were delivered by a 5 cm audio speaker, the centre of which carried a balsa-wood probe holding a thin tungsten wire with a loop at the end. The loop could be positioned over individual spines with the aid of a micromanipulator. The normally open end of each speaker was largely occluded by a steel coverplate to reduce electrical radiation, but which also attenuated sound transients. The speaker was driven by a pulse generator, the output of which was usually shunted by a capacitor to critically damp its ringing and reduce audible clicks.

In experiments that simulated sound-induced substratum vibration (also used to estimate the relative energies involved in auditory reception and mechanoreception), legs were mounted on a sturdy aluminium pillar but with the tarsus resting on a flexible

cantilever beam (see Fig. 7). Movement of the beam was monitored by direct contact with a thin leaf spring to which had been bonded a constantan strain gauge (type EA13060LZ120, MM Inc., Rayleigh, NC). For other tests, a more sensitive silicon beam gauge was used (AE 801, SensoNor, Horten, Norway). Each gauge was calibrated by loading the end with known weights and by deflecting it through measured distances under a microscope. Gauges had adequately flat frequency responses up to the limit tested (5 kHz).

Sound stimuli were delivered from a 15 cm dynamic speaker (Realistic 40-1285C), mounted on a moveable floor stand kept separate from the preparation table to minimize possible vibrational coupling. Unless otherwise stated, the speaker was positioned 70–90 cm from the preparation (usually 80 cm, about 4 wavelengths at 1.8 kHz) to avoid possible near-field particle displacement stimulation of the auditory organ (Tautz, 1979), which, however, turns out not to be a particle displacement detector. The sine wave output of a function generator with an accurate frequency counter (B&K 3011B) powered the speaker. This oscillator was modified so that it could be gated by a pulse generator to produce tone pulses with exponentially shaped leading and trailing edges (time to half-amplitude, about 5 ms), using an AD534KD chip (Analog Devices, Norwood, MA) as an amplitude modulator. This gave useful output levels up to about 10 kHz. Pulse quality was monitored with an analogue oscilloscope from a microphone mounted close to the preparation and was usually recorded by averaging in the peak-channel-hold mode on a digital storage oscilloscope (Iwatsu DS-6411) to recover the pulse envelope, which was otherwise distorted by undersampling. The stimulus pulse started with a variable phase at each presentation, but inspection and controls using phase-invariant pulses from another function generator (Hewlett-Packard 3310B) established that the neural response was not phase-sensitive at any of the frequencies employed. Most experiments were conducted inside an acoustic isolation enclosure about 1 m square lined with sponge-foam wedges, reducing most ambient noise to 20–30 dB below the best response thresholds in the frequency range of most interest (0.7–6 kHz). Frequency tuning curves were compiled by adjusting sound intensity to give a criterion-averaged response at each chosen frequency. Sound pressure levels (SPL, in dB relative to 20 μPa) were measured at each frequency with a Brüel and Kjaer 2203 sound level meter and type 4145 condenser microphone, mounted just to one side of the preparation, by switching to continuous tones of the same intensity. The evoked response became irregular at low SPL and was therefore largely eliminated by superimposition of its positive and negative components when signal-averaging was attempted to improve resolution of 'threshold' responses. This cancellation effect was overcome in some experiments by removing either the positive or negative excursions with a diode clipping circuit, rendering the waveform monophasic, then averaging the remaining response (see Fig. 9B).

Attempts were made to localize the source of neural responses by locally blocking nerve conduction. This was accomplished, reversibly, for 1–2 min after local application of single ice crystals 1–2 mm across to selected points on the surface of the leg or, irreversibly, by local cautery with a small drop of hot Tackiwax applied from an electrically heated wire loop or by local ablation with a tungsten needle.

Directionality of the auditory response was assessed by suspending legs with implanted flexible recording wires at the centre of a 60 mm diameter metal hoop mounted on a vertical pillar. The pillar could be rotated through known angles about the vertical axis by a stepping motor or by hand, moving the leg relative to a speaker a fixed distance away in the auditory far field (usually 70–80 cm away, 4–5 wavelengths at 2 kHz). The 2 mm thick rim of the hoop created no appreciable sound shadow at any position or sound frequency used (see Olson, 1957, p. 21).

In three experiments, the leg on the suspension mount used for directionality tests was placed inside a flattened polyethylene bag which was then perfused with a helium mixture that diffused into the tracheal system of the leg. If the severed end of the coxa is sealed with wax, all neural responses from the leg vanish within about 5 min through asphyxia (the effect is reversible if the stump is reopened soon afterwards); therefore, no attempt was made to use pure helium, although, surprisingly, this seems not to interfere with neural activity in crickets (Paton *et al.* 1977). The bag was inflated with a 80 % He:20 % O_2 mixture (Canadian Liquid Air) at low pressure and perfusion was continued at a lower rate, to obviate adaptation to the hissing sound made by rapid gas flow. To establish the speed of sound (c) in the 80:20 gas mixture, a click emitted from a speaker was conducted inside a plastic pipe which carried two small condenser microphones 1 m apart in its wall, the outputs of which were signal-averaged to obtain the transmission delay. With air in the tube, the value obtained was identical to that measured over the same distance in free-field conditions (346 $m\,s^{-1}$), within 0.6 % of that anticipated from the prevailing temperature and relative humidity. During He–O_2 perfusion of the tube, c increased 1.85-fold to 640 $m\,s^{-1}$, compared with a 2.9-fold change quoted for pure He by Paton *et al.* (1977).

Legs were tested for sound emission from the cut coxal tracheae while parts of the tibial surface were vibrated with a probe made from a small speaker, damped and muffled to reduce sound emission. Provided that weak stimuli were used and the detecting condensor microphone was hermetically sealed to the coxa, externally conducted sound and vibration could be held to levels that did not compromise detection of tracheal sound emission (see Fig. 10).

Behavioural responses to sound were investigated by monitoring movements made by a tethered cockroach held astride a spring-loaded platform (see Fig. 13), displacement of which intercepted the infrared beam of a light-activated switch (Optek type OPB866T55), operated at 15 V. Properly positioned, this device produced a graded low-noise output of 12 mV per micrometre displacement, that was linear for the limited excursions measured.

Results

General observations on the nature of electrical recordings from the leg

Metathoracic legs removed from adults by section distally in the coxa routinely gave useful nerve responses for at least 5–8 h without further maintenance, although sensory background activity rose with time. Spontaneous motor activity was never recorded in isolated legs, except initially when part of a leg was cooled locally with an ice crystal. The

end of the main trachea severed at the coxa must remain open or asphyxia follows within minutes (see Materials and methods). The ultimate cause of neural failure is normally desiccation: in one case, a leg left immersed in water but for the coxal stump still gave neural responses 25 h after its isolation.

Unless the leg is grossly damaged by the recording electrodes, neural responses can invariably be elicited from it, to a large extent regardless of where the recording probes are placed. This is because the leg behaves as an isolated, narrow volume conductor separated into quasi-compartments by higher resistance sections: its structure channels the action currents into one dimension, along the leg. It is easily shown that the electrodes need not come anywhere near the actual nerve bundles for neural activity to be detected. As a consequence, any nerve impulse anywhere in the leg that generates sufficient longitudinal current to exceed recording noise level will be detected, if the two differentially connected electrodes span a significant length of that neurone's axon (S. R. Shaw, D. S. MacIntosh, K. M. Kokic, in preparation). This accounts for the otherwise puzzling observation that stimulation of any large spine on the tibia nearly always gives rise to recorded impulses, which would not be anticipated if electrodes recorded directly, and thus selectively, from axons within nerve 5 in the femur, as is generally supposed for this preparation (e.g. Chapman, 1965; Zill and Moran, 1981).

Mechanoreceptor responses from spines

The extracellularly recorded response to mechanical stimulation of a tibial or femoral tactile spine with a loudspeaker probe is usually complex. A short-latency, initially negative, oscillatory response usually precedes and then apparently intermixes with later biphasic responses that resemble conventional action potentials. Local heat cautery reveals that the biphasic impulses originate locally within the spines, as expected, but that the initial oscillatory response is a field potential originating from the axons of a more remote receptor organ. Surprisingly, this oscillatory, vibration-evoked potential (VEP) usually exhibits an even lower threshold than that of a spine's own bipolar neurone.

Responses to airborne sound

Just as a VEP can be evoked by very weak direct mechanical contact, a similar oscillatory response can be elicited by modest auditory stimuli delivered from a loudspeaker mounted independently from the leg, in the acoustic far field (see Materials and methods). The oscillatory potential is dubbed the auditory evoked potential (AEP), since its identity with the VEP cannot be taken for granted. The signal-averaged response to a shaped tone pip is maximal at 1.3–2.6 kHz and consists of up to 8–9 cycles of regular oscillation that progressively attenuate after the first or second cycle, before disappearing into recording noise (Figs 1, 2). Although adaptation does occur, envelope recordings (Fig. 1) and individual sweeps demonstrate that the response is actually prolonged throughout the tone and sometimes beyond, but that the later oscillations are not as precisely synchronized from one repetition to the next as the first few, so that the later positive and negative components tend to cancel with extended averaging. There was no additional off-response at any frequency tested, provided that tone pulses with slowed rise and fall times were employed (see Materials and methods).

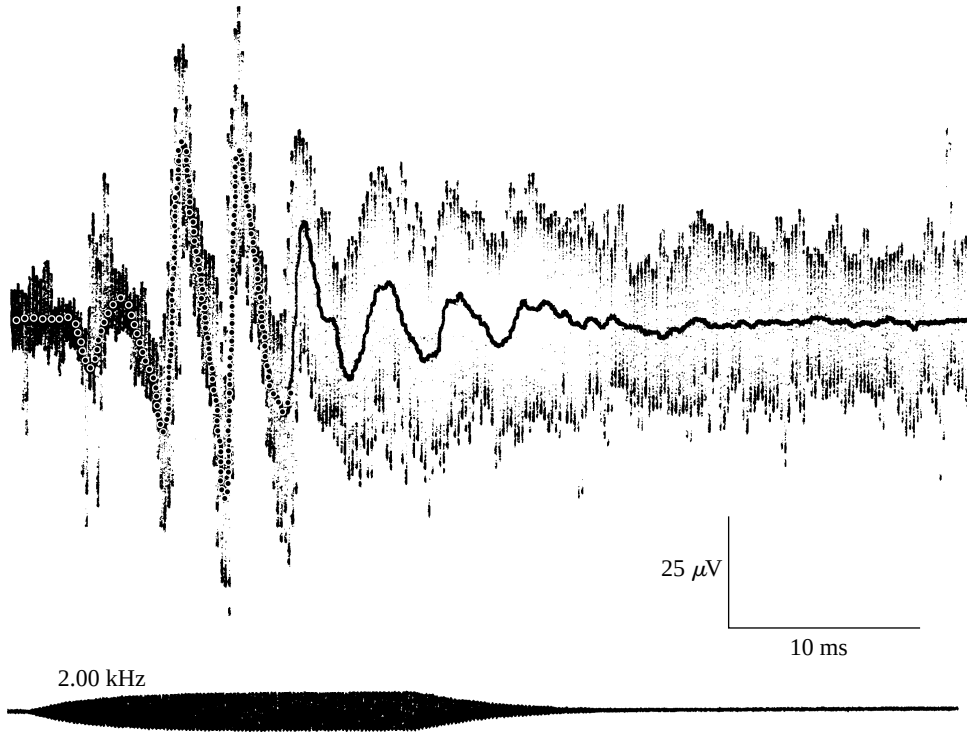

Fig. 1. Averaged auditory response (upper trace) recorded from the metathoracic leg nerve of a cockroach to a brief 2 kHz tone (lower trace) superimposed on the response envelope depicting the most extreme positive and negative voltage excursions recorded in each digitizing bin throughout 64 repetitions. Although the response does adapt, the recorded average (circles and central trace) conceals the fact that considerable activity persists throughout and beyond the stimulus, as revealed in the envelope recording. This later part of the response is largely lost by averaging because its components are not synchronous on the different sweeps, in contrast to the marked synchrony of the first few responses.

The averaged response usually consisted of an initial small negative wave with a minimum latency of approximately 3 ms, followed by a larger negative wave that led into alternating positive and negative oscillations (Figs 1–3). The significance of these components is not immediately obvious. The leg is electrically compartmentalized by high extracellular resistances near the joints, and the small initial wave appears to be a recording of the first cycle of response across the most distal of these at the end of the femur, close to the organ of origin, as a result of a low-resistance shunt pathway established from the most distal electrode through the femur's main blood channel. Subsequent response cycles from this distal site are overshadowed when a much larger negative field from the conducted response reaches a coxa–trochanter resistance barrier across which most recordings were made, 2–3 ms later. This interpretation is supported by tests in which the proximal femur was cooled locally with ice, which would reversibly block conduction there, but would not affect a more distal receptor organ. Cooling reveals that small monophasic responses from a distal site precede each larger response by a fixed

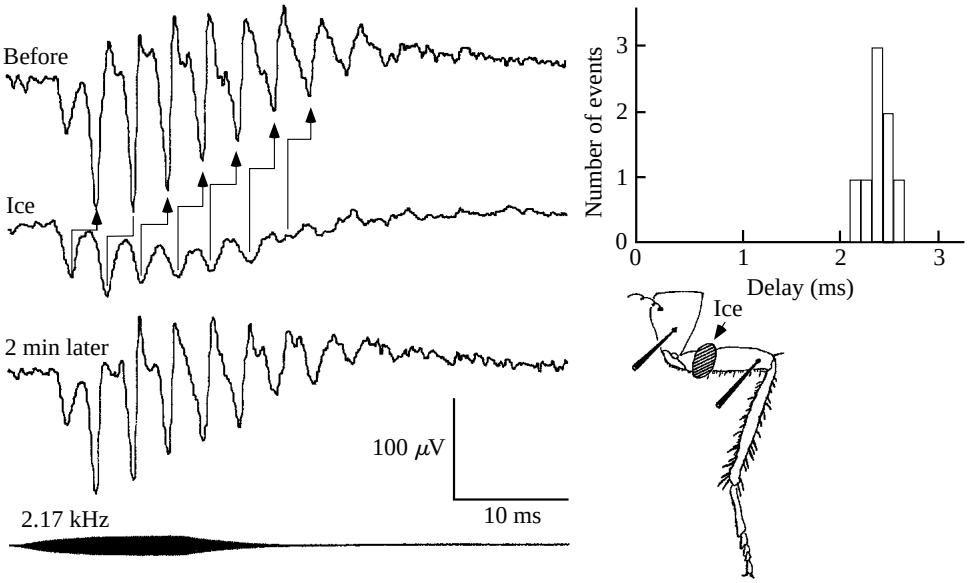

Fig. 2. Averaged auditory response to a 2.17 kHz, 92 dB SPL tone before, during and 2 min after application of an ice crystal to the point shown on the femur. The ice reversibly blocked any responses from being conducted proximal to that point. The large voltage excursions that are abolished by cooling stem from nerve impulse currents generated across a high extracellular resistance at the coxa–trochanter–femur articulation. The smaller excursions uncovered by cooling represent the corresponding impulse currents generated on average 2.3 ms earlier (arrows; histogram) by conduction across a high resistance in the distal femur, just proximal to the subgenual organ in the tibia, from which they originate. Average impulse frequency for the first two cycles of the response was 303 Hz; each trace was signal-averaged 64 times.

interval of about 2.3 ms (Fig. 2). Except for the first response wavelet, the normal response is therefore dominated by the summed currents of conducted nerve impulses. To survive extended signal-averaging, the first few impulses on each repetition must be synchronized in the different contributing receptor cell axons (Fig. 2). The subgenual organ gives rise to 25–26 axons (Fig. 4 of Howse, 1968; Schnorbus, 1971; S. R. Shaw, D. S. MacIntosh, K. M. Kokic, in preparation), raising the question of how this many axons become so effectively synchronized.

Frequency invariance in response–intensity data

Seemingly against the interpretation of the AEP as a compound volley of impulses, the response oscillation frequency f_R remained largely independent of changes in sound intensity over almost the entire range for which oscillation could be measured (Fig. 3A,B). Such invariance is contrary to expectation for a frequency-coded impulse discharge driven from a receptor potential of intensity-linked amplitude, where mean impulse frequency normally changes markedly in proportion to membrane potential (e.g. Fuortes, 1959). Response amplitude and latency functions were similar to those found in other receptor systems, however, increasing and shortening, respectively, as intensity

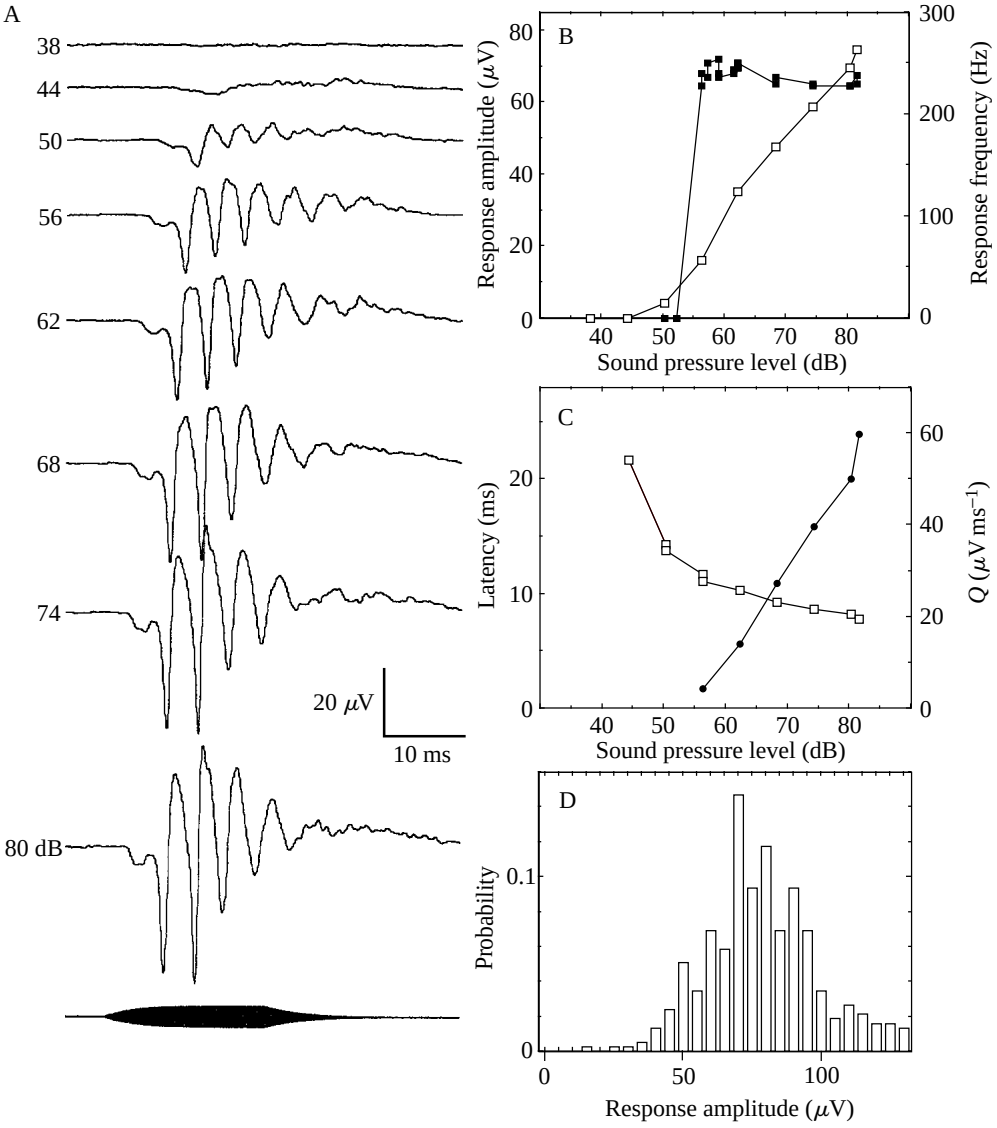

Fig. 3. Response *versus* sound-intensity relationships for the auditory response. (A) Averaged recordings (N=256 each) for 6 dB sound pressure increments at 1.78 kHz, starting from 38 dB (no response; detection threshold was 42 dB SPL at this frequency). (B) The amplitude of the first large negative peak (open squares, left ordinate) increases with SPL, but the effective response frequency f_R averaged across the first two response cycles remains almost constant, near 250 Hz (filled squares, right ordinate). (C) Response latency measured from the beginning of the tone to the first negative peak decreases progressively with increasing SPL (squares, left ordinate), while factor Q, describing impulse shape, rises sharply (filled circles, right ordinate). (D) A probability histogram for 375 consecutive presentations of an identical 2.02 kHz tone from a different preparation, shows large response amplitude fluctuations recorded during a time window admitting just the first major negative peak of the response.

increased (Fig. 3B,C). Because the response is formed from the individually invariant, conducted impulses summed from the axons of up to 26 sensilla, the increase in amplitude in Fig. 3B must reflect a progressive recruitment of more axons at higher SPL. This, in turn, might imply differing thresholds in different sensilla, leading to invariant, quantized steps in response amplitude as stimulus intensity increases, but clear evidence of quantization was not obtained, perhaps because of the masking effect of background firing from other axons in the leg. Alternatively, the thresholds of all the sensilla might be similar, but vary over time according to internal noise or threshold fluctuation at the site of impulse initiation, in which case the growth of the response would reflect the increasing probability of more axons reaching threshold at higher SPL. The suggestion that noise or threshold fluctuation is a contributing factor is indicated by the very wide dispersion of the peak amplitude recorded on successive presentations of an identical stimulus (Fig. 3D), but f_R remained invariant despite this.

A possible explanation for this behaviour is that the organ, its immediate structural environment or each receptor cell is (or contains) an intrinsically tuned, damped resonator energized by sound and that the tuning of impulse frequency at about 300 Hz merely reflects an antecedent, tuned, oscillatory receptor potential. If internal noise also contributes to whether a sensillum reaches threshold, invariance of f_R would still be anticipated, but the dispersion of impulses around the mean time of occurrence ought to increase at lower SPL. This is the likely explanation for the relative broadening of the shape of the recorded impulses as SPL is lowered, evident in Fig. 3A, which can be quantified conveniently by a quality factor Q (defined as impulse amplitude divided by impulse half-width): Q rises sharply with intensity (Fig. 3C).

The interval between compound impulses remained practically invariant from one cycle of oscillation to the next (Figs 1–3). This again fits the idea of a resonating system, but is contrary to expectation for adapting impulse discharges, including those recorded from cockroach legs where the instantaneous discharge frequency drops progressively throughout a burst, concomitant with adaptation (Chapman, 1965). The striking invariance of f_R with both time and stimulus intensity has also been observed in cricket legs (S. R. Shaw and D. S. MacIntosh, unpublished results), but appears to have been missed in previous accounts of both auditory and ‘vibration’ organs, presumably because these have used single-cell recordings which fail to reveal such population spike effects.

Independence of response frequency and stimulus frequency

Although both stimulus and response were oscillations, no correspondence between their frequencies was expected or observed. In each preparation measured, response frequency f_R was essentially constant and independent of stimulus frequency in the range that could be readily tested (see Fig. 8B). When determined by averaging over the first two response cycles, f_R ranged from 250 to 370 Hz in different preparations at room temperatures (where recorded) of 21–23.5 °C (mean 300 ±28 Hz, S.D., N=80; Fig. 4). During a recording session f_R often changed slowly, perhaps reflecting desiccation (Chapman, 1965) or a slow decline in metabolic competence. As found in single cells of the crista acoustica of bushcrickets (Oldfield, 1988), experimental changes in temperature

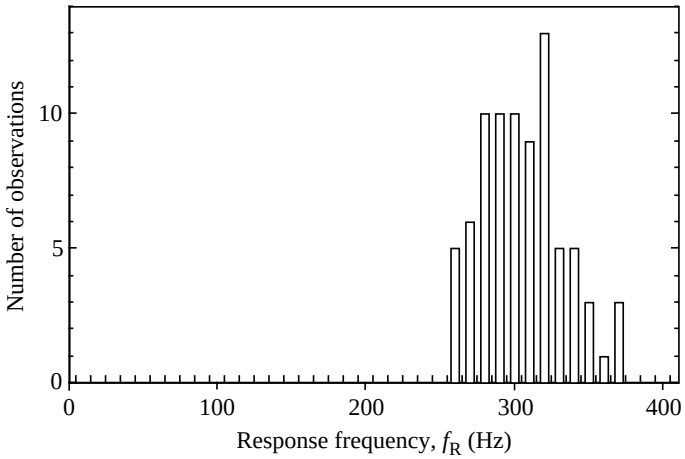

Fig. 4. Variation in response oscillation frequency (f_R) observed in representative records taken from 80 metathoracic legs. f_R was obtained by averaging the peak-to-peak time between the first two large response cycles and converting this to an instantaneous frequency (mean 300±28 Hz, S.D.).

affected the AEP markedly, f_R increasing with a Q_{10} of approximately 2 (S. R. Shaw, unpublished results). Because the precise ambient conditions were not recorded for many experiments, variations in room temperature could have contributed to the dispersion evident in Fig. 4.

Controls against artefacts and audioreception by hairs

The oscillatory response to sound could be completely and reversibly abolished by placing a sheet of thick polystyrene foam in front of the speaker. This demonstrates (1) that the response is not an artefact caused by leaking electromagnetic radiation (which would be unattenuated by polystyrene); (2) that vibration conducted from the speaker's mounting down into the floor and up again into the experimental table is also negligible; (3) that, to a first approximation, lateral sound leakage could also be neglected, although it was audible to the experimenter. The response is therefore caused by airborne sound propagated in a direct line to the leg.

Hairs on caterpillars and on orthopteran cerci (Tautz, 1979; Pumphrey and Rawdon-Smith, 1936) respond to low-frequency sound under near-field conditions by viscous coupling to air particle displacement rather than to the pressure component of the stimulus. Cockroach legs also bear numerous mechanosensory hairs, but none of the experiments here involved near-field stimulation that might have stimulated them. Moreover, completely smothering isolated legs with a thick coat of petroleum jelly had only a small immediate effect on the AEP, and this could be attributed to a few decibels attenuation of the 2 kHz test sound by the coating. A similar (reversible) small reduction has been obtained in several tests by immersing parts of the leg in water. After application of petroleum jelly for several minutes, the AEP declined through anoxia caused by tracheal blockage. The sound receptor must therefore be an internal organ, not a set of

external hairs. The response to sound persisted unaltered regardless of whether the leg was suspended in air or water and whether it was left largely free or fixed with wax in various ways to rigid supports. This makes it implausible that the response is caused by general vibration of the leg's surface in the sound field, one suggestion Autrum (1941) made to explain a supposed indirect response to low-frequency sound obtained from the tettigonid subgenual organ (see Discussion).

Site of origin of the VEP and AEP

The origin of the recorded responses has been narrowed down in experiments using heat cautery, reversible local cold block with single ice crystals applied at different positions (Fig. 2), conduction delay projections and current-source-density estimates, to a point just distal to the femoro-tibial joint. Neither VEP nor AEP was abolished by any treatment over large regions of the tibia until applied close to this point, although the shape of the averaged response could be affected.

Schnorbus (1971) and earlier authors (see Dethier, 1963) describe three groups of chordotonal sensilla at this location, the largest and most vibration-sensitive of which is the fan-shaped subgenual organ (SGO). It is supposedly slung eccentrically in one haemolymph channel between the opposite walls of the tibia (Schnorbus, 1971; Howse, 1968), although we have found that the organ is suspended between the wall and the large tracheal expansion at this site (Shaw *et al.* 1992; S. R. Shaw, D. S. MacIntosh and K. M. Kokic, in preparation), as depicted also by Howse (1967). Schnorbus (1971) pinpoints an insertion of the SGO on the tibial forewall, just proximal to the first 'dorsal' tibial spine, D1. When a tungsten needle was used to make a highly localized ablation through a small hole at this site in three tests, both the VEP and AEP were abolished irreversibly (Fig. 5), providing the most explicit evidence for involvement of the subgenual organ complex in generating both responses. It might be objected that local ablation merely destroyed the axons of an organ lying even more distally, but the cautery and cooling tests on the tibia eliminate this possibility.

These results point to the subgenual complex as being the origin of both the AEP and the VEP, suggesting that it may function, at least in part, as an auditory organ.

Cross-modal interaction between sound and vibration

Because the recordings were extracellular, it could be argued that the responses to sound and vibration come from the separate axons of two different, nearby sense organs, but the available evidence weighs against this. First, locally destroying the site of the SGO by ablation removed both the AEP and the VEP (Fig. 5). The two responses therefore must either originate in the same structure or in two organs intimately associated at the same site. In the latter case, cross-modal adaptation would not be expected to be effective, but the opposite is observed: if a sound pulse is made to precede a vibration stimulus or *vice versa*, the second response is markedly attenuated by the first (Fig. 6), just as is observed with consecutive tone stimuli (see Figs 8, 9) or mechanical stimuli (data not shown). Results with continuous adaptation (next section) support this finding and, together, implicate the same receptor cells as the origin of both the

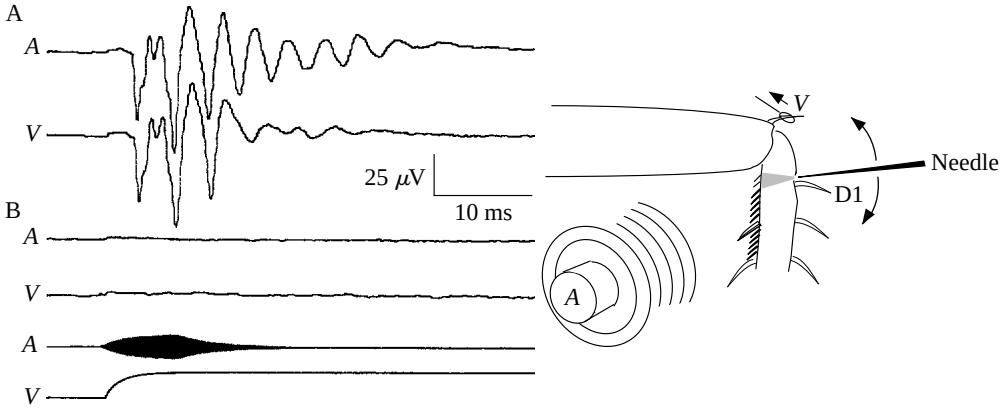

Fig. 5. Effect of local ablation on the auditorily (*A*) and vibrationally (*V*) evoked responses. (A) Averaged AEP response to a 2.02 kHz tone from a speaker 80 cm from the leg, compared with a VEP of matched initial amplitude from the same preparation, produced by deflecting the distal femoro-tibial spine by an amount subthreshold for its own neurone, using a wire loop attached to a speaker. (B) Responses to identical stimuli disappeared after destroying the site of the SGO by probing through the wall of the leg just above the D1 spine with a tungsten needle, as shown at the right. Both AEP and VEP are abolished, as expected if they originate in a single structure. Each trace is the average of 128 repetitions. The two lowest traces are stimulus markers for *A* and *V*.

vibrational and the auditory responses. The obvious alternative, that adaptation is mechanical and occurs external to the subgenual complex, is unlikely in this case (see Discussion).

Is sound detected via *ground vibration?*

The similar properties of the VEP and the AEP might be explained if energy contained in one modality were simply channelled into the other. Moran and Rowley (1975), citing earlier authors, indicate that cockroaches can detect sound because it induces substratum motion which is picked up by the vibration-sensitive SGO, a possibility that Autrum (1941) first considered and felt unable to reject, because of uncertainty about the level of induced vibration in his experiments, despite his efforts to reduce this to a minimum. Because of its expected inefficiency, this is a physically implausible suggestion for most potential substrata for all but the loudest sounds, but it is difficult to refute convincingly in principle because the SGO is so sensitive to vibration (Autrum and Schneider, 1948; Schnorbus, 1971), and the idea appears never to have been examined critically. It was tested here using an approach opposite to Autrum's: an isolated leg was mounted atop a short rigid pillar, and the tarsus was attached to a flexible support with well-understood vibrational properties, a cantilever beam extending from the pillar (Fig. 7A). Reproducible, small-amplitude, self-damped oscillations of the beam at its fundamental frequency were induced by brief taps from a metal probe attached to a small speaker. The voltage drive to this was adjusted to register a strong VEP response (recorded through flexible 75 μm diameter wires implanted in the leg, to uncouple this mechanically from other apparatus). In this configuration (the most sensitive for exciting the SGO according

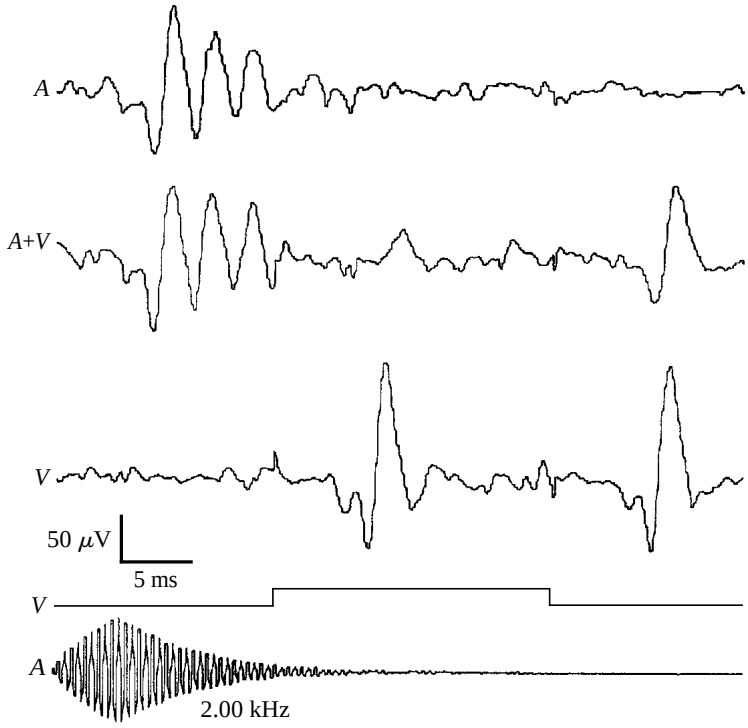

Fig. 6. Cross-modal adaptation. A 2 kHz tone sounded alone produces an adapting oscillatory AEP (trace *A*). A weak mechanical deflection of a tibial spine by itself produces a large transient VEP at both on and off (trace *V*). When the tone precedes the mechanical stimulus (*A+V*), the VEP is transiently abolished, indicating that adaptation to one modality interferes with the response generated by the other, as would be expected if the same sensory cells were involved in generating both AEP and VEP.

to Schnorbus, 1971), a large oscillatory output emerged from a strain gauge in direct contact with the beam (Fig. 7A,B). By contrast, when a sound stimulus was adjusted to evoke an AEP response which matched the VEP amplitude, no induced vibration could be measured in the averaged strain gauge recording (Fig. 7B), indicating that the response to sound is not mediated through sound-induced vibration of the substratum, the cantilever beam. This conclusion would hold even if some undetected higher harmonic were the effective stimulus, because the power distribution between harmonics remains independent of the source of excitation, so that the fundamental would still appear in the strain gauge record. No induced movement of the pillar itself or of the beam extending through the other side of it (Fig. 7A) could be detected with the strain gauge, with either the vibration stimulus or the tone.

The result was complemented by evoking a VEP response as before but during presentation of a continuous 1.68 kHz tone. The tone almost completely abolished the VEP, but the response from the strain gauge was identical with or without the sound pulse (Fig. 7C). This indicates that adaptation to sound is not mediated by substratum vibration either, even for this rigid system made of metal that transmits imposed high-frequency

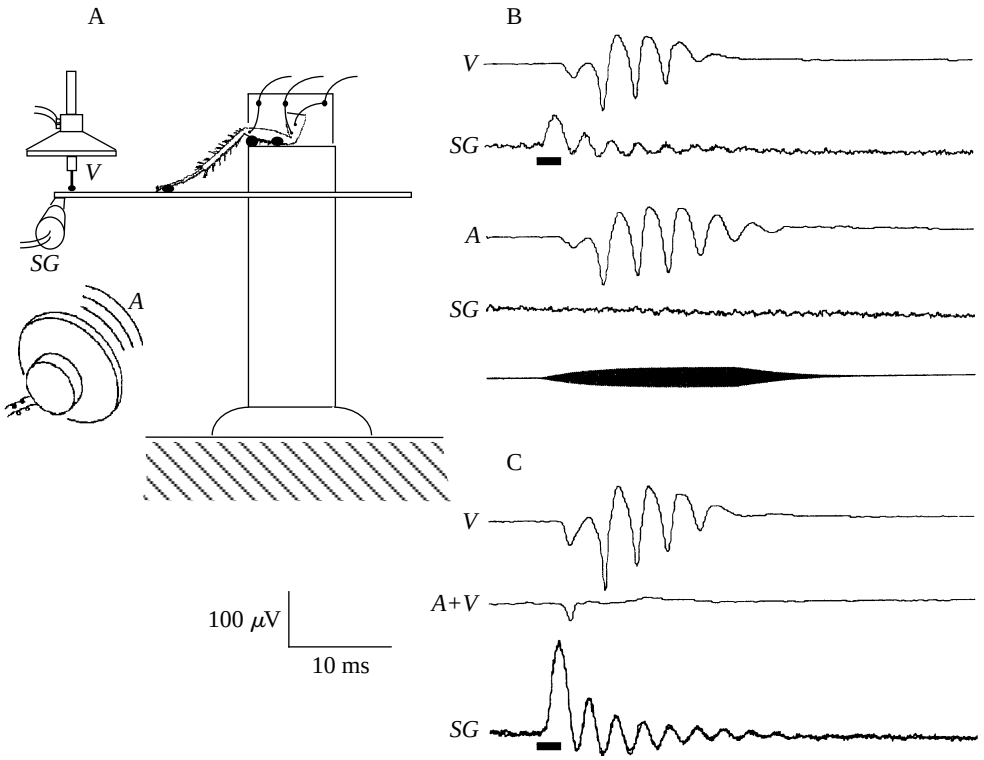

Fig. 7. A test of the hypothesis that substratum vibration mediates the auditory response of the cockroach leg. (A) A metathoracic leg with implanted fine recording wires was fixed to a rigid tower with wax, and its tarsus was attached to a flexible cantilever beam extending from the tower. (B) Vibratory (*V*) and auditory (*A*) stimuli were matched to give similar responses, while the motion of the beam was monitored with a strain gauge (*SG*). The beam responded with damped oscillations after a brief tap (bar) near its end (*V*, *SG*), but no movement was detected during the response to the 1.68 kHz tone (*A*, *SG*), showing that the auditory response is not mediated as vibration induced in the beam by the sound. (C) The response, *V*, to a larger impulsion of the beam is strongly adapted during a continuously presented tone (*A* +*V*), but the strain gauge recording is unchanged.

vibrations quite well. The success of this cross-modal adaptation again suggests that the same receptor cells are activated by both vibration and sound.

Auditory tuning and threshold

If the cockroach subgenual complex is an effective ear, it might display tuning to some optimum frequency, as do other insect auditory organs, and have a usefully low threshold for airborne sound at this best frequency. A few tuning curves were therefore compiled (Fig. 8A) by adjusting tone pulses of different frequency to evoke averaged responses of similar amplitude (Fig. 8B). These will be described more fully elsewhere in connection with the mechanism of sound entry to the leg. The centre (best) frequency varied from 1.3 to 2.6 kHz in different metathoracic legs, comparable with values obtained by Schnorbus (1971) using vibratory stimuli. Subsidiary peaks or inflections were usually found

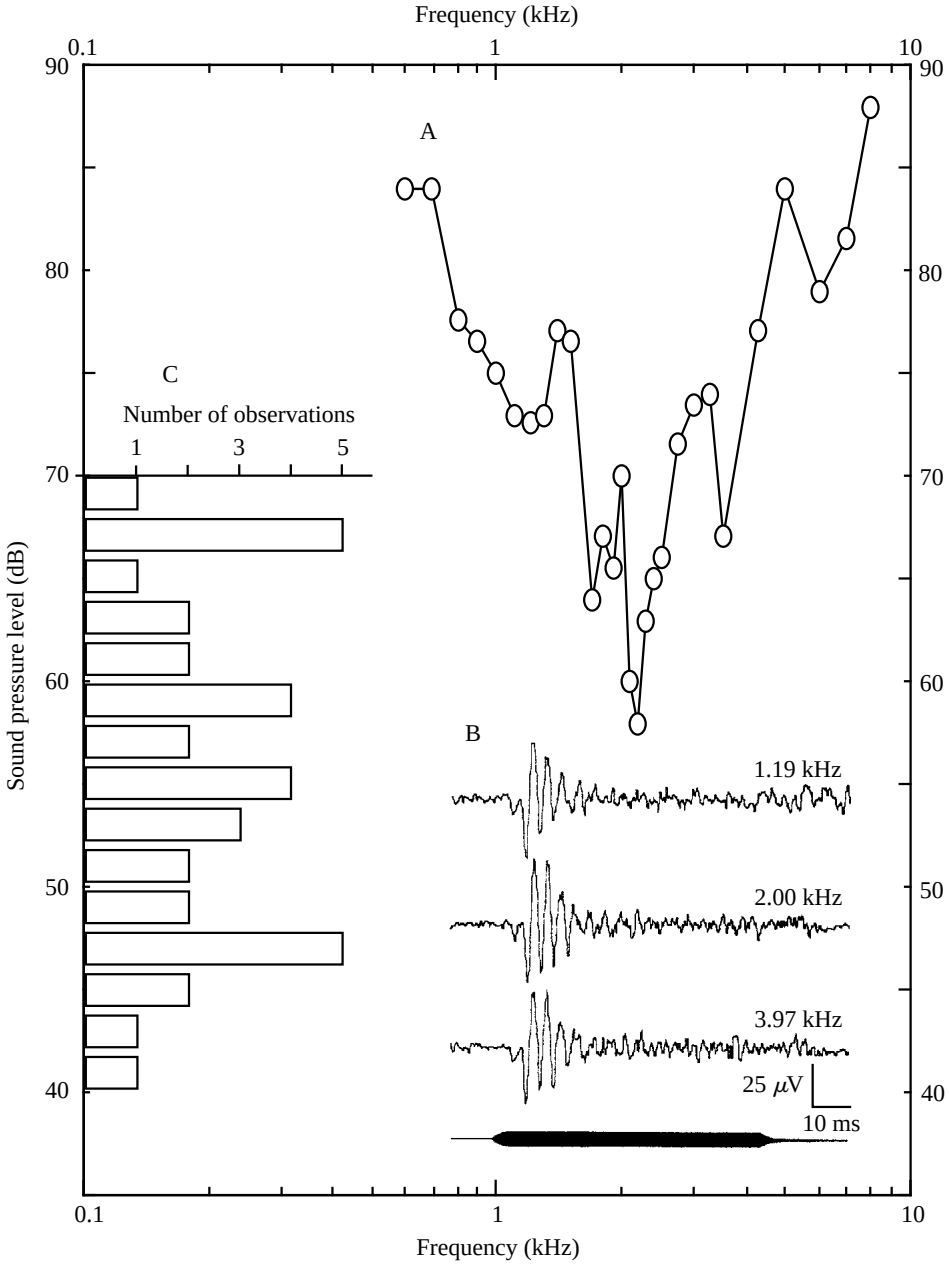

Fig. 8. (A) Auditory tuning curve determined by adjusting the stimulus intensity to obtain a criterion averaged response a few decibels above detection threshold at each frequency. Several subsidiary peaks are evident on both sides of the centre frequency at about 2.2 kHz. (B) Suprathreshold auditory responses (32 averages each record) from the same preparation to different frequencies, using tones of 1.19, 2.00 and 3.97 kHz. When adjusted in intensity, the three responses are practically indistinguishable; a response univariance expected if the same cell type mediates all the responses. (C) Distribution of auditory response thresholds (in dB SPL) measured close to the appropriate centre frequency for each of 37 metathoracic leg preparations.

(Fig. 8A), again as reported by Schnorbus (1971). The sound pressure level needed to evoke a threshold response at the best frequency varied considerably in different preparations, from 41 to 68 dB (mean 55±8 dB, S.D., *N*=37; Fig. 8C). The lower values are comparable to or better than those of accredited auditory receptors in insects, although some have been found that are more sensitive. Values might have been even lower had estimates been made using single-cell recording, since Michelsen (1971) reported a consistent difference of about 11 dB in a comparative study on acridids. Some of the values in Fig. 8C may be artefactually elevated by up to 15 dB for a different reason: initially, it was assumed that the acoustic system of the leg would be non-directional (see below), and no attempt was made to orient it consistently with respect to the sound source in the early experiments.

If the subgenual organ contains groups of receptors tuned to different frequencies that can also be excited independently, it should be possible to reveal this in the field potential recorded from the leg by selectively adapting at one frequency and then testing at the same and at other frequencies. In metathoracic legs, this approach produced no obvious differences. All test responses were strongly and similarly attenuated by the first tone, regardless of test frequency (Fig. 9A), suggesting that there may be only one type of auditory receptor in each SGO or that any differences present are overshadowed by a common mechanical response.

An additional small-amplitude response to low sound frequencies around 250–300 Hz was recorded briefly from a few legs. This was distinguished by its much longer latency, by a different frequency of intrinsic oscillation and, in the one case tested, by independence from adaptation at the centre frequency of the main response of the SGO (Fig. 9B). This low-frequency response presumably comes from a different organ system in the leg, but its origin has not been pursued.

Sound emission from the leg

If airborne sound reaches the SGO *via* a different pathway from contact vibration, as the results above suggest (Fig. 7), it seems surprising that the auditory tuning function (Fig. 8) resembles the vibrational tuning curves of Schnorbus (1971). The tracheal system's acoustic transmission characteristics might be expected to shape auditory tuning, but not the vibration tuning curve. The observed similarity might be explained if transduction for both modalities shared a common mechanical basis, but as deformation of the tracheal system rather than as vibration of the leg or substratum, thus inverting Autrum's (1941) original thinking. A second reason for interest in the mechanics of vibration detection is that the evolutionary advantage of linking of the SGO to the tracheal system may have been a consequent increase in sensitivity, by virtue of the compressibility of the attached air spaces (see Discussion). Both lines of thought thus predict some 'acoustic' outcome for vibrational stimuli in the form of pressure changes inside the tracheae, if contact vibration does distort the tracheal system significantly.

Evidence from four experiments confirmed this prediction. When the tibial surface was stimulated lightly by a vibrating probe in conditions which eliminated both artefactual leakage of airborne sound and substratum vibrations, sound emission could be recorded by a microphone connected to the coxal tracheae (Fig. 10). Vibration applied to the tibia

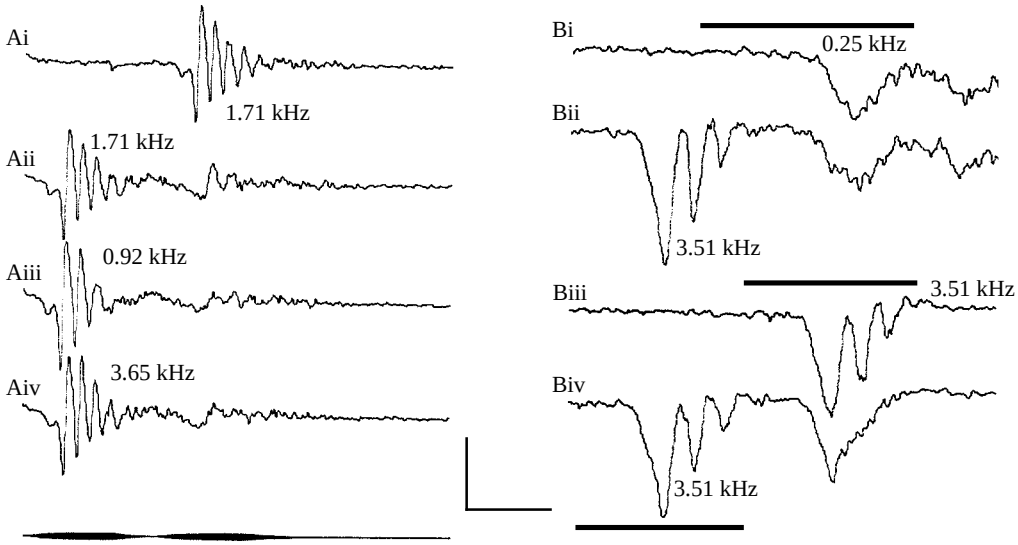

Fig. 9. (A) The averaged response to a 1.71 kHz tone presented alone (i) is strongly and similarly reduced by preadaptation with an earlier tone presented at the same frequency (ii), at a lower frequency (iii) or at a higher frequency (iv), provided that each produces a similar-sized initial response. (B) From a different preparation, the slow long-latency response to a 254 Hz tone (upper bar, i), probably from a different organ system in the leg. It is unaffected by preadaptation with a 3.51 kHz tone (lowest bar, ii) that produces an oscillatory response typical of the SGO. The response to a second 3.51 kHz tone (middle bar, iii), by contrast, is markedly affected by preadaptation at the same frequency, (lowest bar, iv), as expected. The preparation was a chronically dissected semi-intact cockroach. Covering the cerci with petroleum jelly did not affect the responses. The waveshapes in B were rendered monophasic by passage through a diode clipping circuit before averaging. Scale bars: 40 μV, 20 ms in A; 20 μV, 10 ms in B.

produced the largest signal when the probe was applied distally at points where the large terminal air sac approaches or contacts the cuticular surface, suggesting that a prototype tympanal function for this region needs to be considered. The ‘sound’ signal does not reach the coxa microphone directly as a pressure change in the blood, because shunting this pathway completely by cutting open the femur wall has no effect on either the microphone signal or the neural response (Fig. 10).

Auditory directionality

It was thought originally that the response to sound would show no directional component, because response directionality in cricket ears is believed to depend upon interference between two or more sound inputs to the leg (Larsen *et al.* 1989), whereas the cockroach has only one obvious input, the tracheal pathway running into the leg. Nonetheless, when a leg was rotated at a fixed distance relative to a far-field sound source, the auditory response showed marked directionality, with differential maximum–minimum values up to 15 dB (approximately 6:1) being usual near the centre frequency (N=20). Response profiles were most often cardioid, with sensitivity being

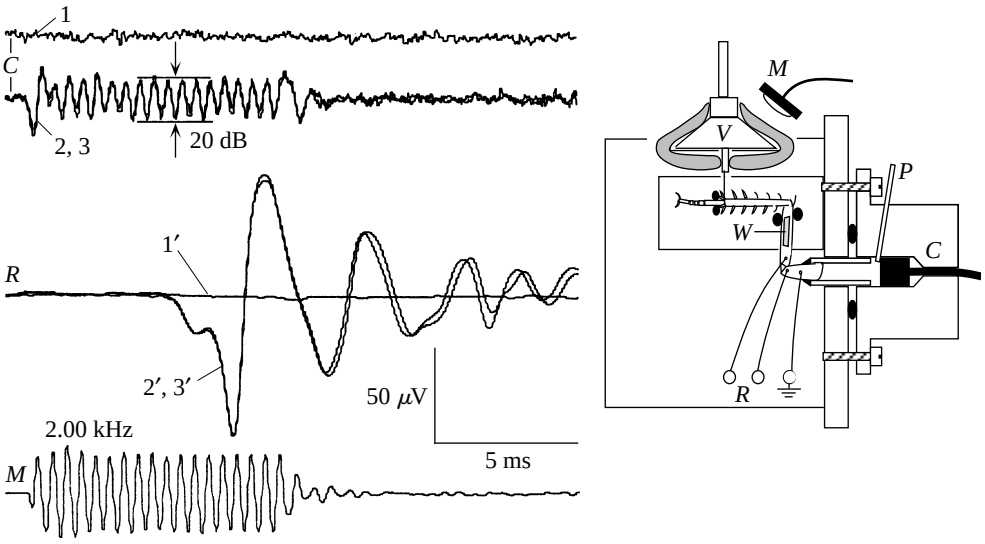

Fig. 10. Airborne sound is emitted from the coxal tracheae when the tibial cuticle is vibrated lightly. Right: plan view of apparatus, not to scale. A probe extending from a small sound-muffled speaker (*V*) is pulsed at low amplitude with a phase-invariant sine wave, vibrating the tibial surface lightly just proximal to tibial spine PD4. The leg is immobilized with wax and its coxa waxed into a tube that is hermetically sealed to condenser microphone, *C*, inside a plastic block. A narrow air access pipe, *P*, was closed during measurements. Three implanted 75 μm diameter wires record the SGO's response (*R*). Left: during application of a 2 kHz vibration (lowest trace), a somewhat distorted sound emission is detected by microphone *C*, equivalent to about 20 dB SPL in free-field conditions (traces 2, 3). This remains unaltered if a large window (*W*) is cut in the femur wall to shunt any pressure change transmitted in the blood (traces 2, 3, before and after cutting). The corresponding neural response *R* is also unaffected by the cut (traces 2′, 3′). If the probe at *V* is withdrawn a few millimeters and made to vibrate the support carrying the leg, both responses disappear (traces 1, 1′), showing that neither airborne sound leakage nor vibration of the apparatus is responsible for either the acoustic pick-up or the neural signal. Movement of the probe is monitored indirectly by acoustic leakage to a nearby high-gain microphone, *M*. Each trace is signal averaged 256 times.

highest when the cut end of the coxa pointed directly away from the sound source (Fig. 11). Directionality is frequency-dependent and its origin is currently under investigation, but it appears to be caused, at least in part, by interference between sound entering the leg at different points, with the severed tracheal system being the most important at the centre frequency (Shaw *et al.* 1992; S. R. Shaw, K. M. Kokic and D. S. MacIntosh, in preparation).

It is not known where sound enters the tracheal system *in vivo*, but the ipsilateral first abdominal (A1) and second thoracic (S2) spiracles are likely to be the most important sites for the metathoracic leg. The tracheal system in *P. americana* is said to be comparatively simple (Baudet and Sellier, 1975), but there is no published description of it. Our own mapping reveals that the SGO is reached by two parallel, cross-connected tracheal paths. The larger-diameter (and presumably acoustically dominant) of these has its shortest path and expected major input from A1 and a longer connection to S2. Ventral

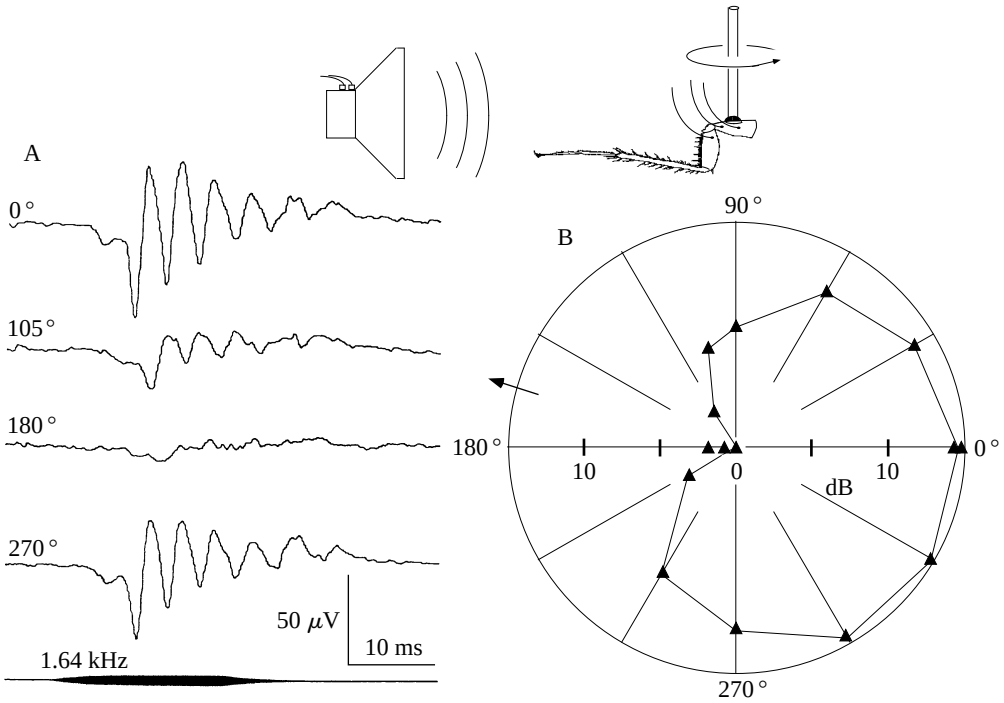

Fig. 11. (A) The averaged auditory response exhibits a marked directionality to a tone presented near the best frequency, when an excised metathoracic leg is rotated relative to a speaker 80 cm away (see inset). The rotational axis shown corresponds roughly to the 'pitch' plane of the animal in a normal horizontal stance. (B) In the same test, the relative auditory sensitivity varies in a cardioid fashion by about 15 dB, compiled using a calibration curve of response amplitude *versus* intensity obtained near the most sensitive position, 330°. The arrow indicates the orientation of the leg at which the cut coxal surface directly faces the speaker.

anastomoses to the contralateral leg occur but are small and branch from the longer, narrower pathway and are therefore likely to be of little acoustic importance. This parallel pathway originates at S2 but also receives a small branch from A2.

Removing the leg from the body is therefore unlikely markedly to distort the measured directionality. According to the current pressure-gradient interference model, directionality depends partly upon the difference between the internal and external acoustic pathlengths (Michelsen, 1971; Larsen *et al.* 1989). The shortest internal path (to A1) adds only 1.7 mm extra in the intact cockroach (about 9 % of the internal pathlength). A change of only about 1 dB in the differential sensitivity is predicted from this extra length, using a two-input model and assuming that the second input lies distally on the tibia. It would be preferable to confirm this experimentally by measurements of directional sensitivity in legs with more complete tracheal systems, but efferent activity in large motor units usually obscured sensory impulses (in undissected preparations with nerve 5 still connected to the metathoracic ganglion). In two acute preparations consisting of the leg attached to its metathoracic segment and the two neighbouring segments and

where the tracheal system with its spiracles was locally intact, obvious directional sensitivity was still observed.

Auditory responses from other legs

Nearly all recordings were made from metathoracic legs, but subgenual organs are found in all three pairs in several insect orders (Dethier, 1963). Exploratory recordings were therefore made from meso- and prothoracic legs. All legs gave qualitatively similar field potentials in response to presented tones. The oscillation frequency was somewhat higher in the more anterior legs, but still fell within the range found for metathoracic legs. One prothoracic leg was optimally tuned to about 3.7 kHz, higher than for any metathoracic leg, but in the absence of a detailed comparative study of tuning the possibility of systematic differences between legs remains an open question.

Optimum response mode: comparison of the energy needed to evoke vibrational and auditory responses

Sound and contact vibration are normally quantified using different units of measurement, but if the energy input needed to produce some criterion response could be determined for both modes of excitation, a comparison of the relative sensitivities could be made in common units.

The most useful comparison for this purpose arose from the simulated substratum vibration experiment described above (Fig. 7), in which the stimulus was delivered in a configuration that had resulted in the lowest threshold for vibrational input (Schnorbus, 1971). The displacement experienced by the tarsus, y_1, could be estimated knowing the distance from the pillar to the tarsus, x_1, by measuring the displacement y_2 at the end of the beam (x_2) with the calibrated strain gauge, according to the expectation for a cantilever beam supported rigidly at one end (Duncan, 1954) that:

$$\frac{y_2}{y_1} = \left(\frac{x_2}{x_1}\right)^2 . \tag{1}$$

The value of y_1 at the leg so estimated during an averaged suprathreshold response (Fig. 7B) was 0.47 μm. The force F_1 applied at the tarsus was calculated from that measured at the end of the beam, F_2, since, according to Duncan (1954):

$$y = \frac{Fx^3}{3EI}, \tag{2}$$

where E is the modulus of elasticity and I is the moment of inertia of the beam. For the two locations x_1 and x_2 on the beam:

$$\frac{F_1}{F_2} = \frac{y_1}{y_2}\left(\frac{x_2}{x_1}\right)^3 . \tag{3}$$

From equation 3, the force F_1 at the position of the tarsus would have been 0.94 mN for the first full-amplitude deflection. The vibrational work W_v done during the first (largest) stimulus cycle can then be estimated as force multiplied by distance moved:

$$W_v = F_1 y_1 , \tag{4}$$

giving 8.8×10^{-10} J for the work done.

Comparison with the acoustic input is possible because the sound pressure (p) outside the leg for an approximately matched response (Fig. 7B) is:

$$p = p_0 10^{b/20} , \tag{5}$$

where b is the measured SPL for a similar response (98 dB) and p_0 is the reference sound pressure of the calibration, 20 μPa. As pressure p is force applied per unit area, F_A/A, the approximate acoustic equivalent of equation 4 becomes:

$$\begin{aligned} W_A &= 6(F_A/A)\,Ad \\ &= 6pAd , \end{aligned} \tag{6}$$

where F_A is the acoustically derived force acting to generate a 'tympanic' movement d over one half-cycle of the auditory stimulus; the factor-of-six multiplier reflects the empirical finding that a minimum of three complete sound pressure cycles is required to produce a full-sized initial response. To evaluate equation 6, the area A over which pressure changes act to produce the AEP needs to be known. Near the centre frequency, about 1.8 kHz, sound enters most effectively *via* the cut ends of the tracheae in the coxa and only secondarily through a part of the wall of the rest of the leg, mostly in the tibia, at about 30 % relative effectiveness (S. R. Shaw, D. S. MacIntosh and K. M. Kokic, in preparation). Because the surface area of the tibia (30±2.1 mm^2, S.D., N=15 legs) is much easier to estimate than that of the tracheal system, this measure was used for calculation and was weighted to compensate for an estimated 30 % efficiency. The distance d moved by the tympanal equivalent lies below the resolution of our current measurement method (<0.5 nm), so a value of 0.4 nm is adopted provisionally, although this would be large by comparison with estimates for other insect auditory systems (Michelsen and Nocke, 1974). The calculated auditory work done is then less than 3.9×10^{-13} J, about 2300-fold less than than that calculated for the vibrational input. However, the latter was delivered at the beam's measured fundamental resonance frequency of 336 Hz, whereas Schnorbus (1971) found that the vibrational sensitivity of the SGO at this frequency is about 300 times less than at its best frequency around 1.5 kHz. Correcting for this reveals that the energy requirements for sound and vibrational inputs only differ by a factor of eight (higher for vibration), well within the uncertainties for some quantities used in the calculation, in particular A and d. However, other observations suggest that area A cannot be the entire tibial surface, although its dimensions are not known. Any correction for this would widen the eightfold gap even further in favour of the auditory input.

The origin of response resonance

The apparent response resonance at approximately 300 Hz could have several possible origins (see Discussion), one of which being that an air mass contained in an expansion of the tracheal system is induced to act as a Helmholtz resonator at this low frequency (Morse, 1948). We have found four large tracheal expansions, two shunting the main tracheae and two in series with them, one of which is attached to the SGO itself (S. R.

Shaw, D. S. MacIntosh and K. M. Kokic, in preparation). This level of complexity precludes the use of any simple acoustical model to describe the system to predict whether part of it could act as a resonator, and at what best frequency, but a general prediction is possible. For a single, simple Helmholtz resonator chamber, the resonance frequency f_1 (Morse, 1948, equation 23.5) is:

$$f_1 = \frac{1}{2\pi}\sqrt{\frac{c^2 s}{lv}}, \tag{7}$$

where s, l and V define the dimensions of the expansion and its access pathway, and c is the velocity of sound. For more complex series of expansions in series or in parallel, related expressions define the cut-off frequencies (Morse, 1948), so that frequency tuning in all these cases should be directly proportional to c. If the hypothesis of direct tracheal resonance is correct, as c is varied, f_1 should change in proportion. In three preparations, c was increased 1.85-fold by perfusing the leg with a mixture of 80 % helium and 20 % oxygen. There was no detectable change in response frequency f_R, only a small phase retardation which disappeared when the leg was perfused with air again (Fig. 12). The invariance of f_R in He–O_2 clearly speaks against identification of the 300 Hz oscillation with any direct tracheal resonance or tuning at this frequency, but does not rule out selective tuning of the tracheal acoustic transmission line to higher frequencies around 2 kHz, which other observations support (S. R. Shaw, unpublished work).

Behavioural observations on sound detection by cockroaches

If the auditory properties described so far are not just epiphenomena, some behavioural correlate might be expected. A sensitive auditory system might be involved in interactions with conspecifics, perhaps during mating displays, or it might function as part of an acoustic warning system. The first seems unlikely, because a sensitive microphone lowered into a large colony at night in the dark when most insects were active picked up very little noise and none of an intensity sufficient to activate the SGOs, in the bandwidth monitored up to 12 kHz. This, and the fact that no significant sounds were audible even though normal human auditory threshold near 2 kHz is at least 40 dB lower than that of the metathoracic leg, supports this cockroach's reputation as a silent species (Roth and Hartman, 1967).

The possibility of a sound alarm system was initially examined by aiming high-intensity tone pulses from short range at individuals roaming inside the colony box at night, but no reliable changes in behaviour were observed under any stimulus permutation tried. Many individuals responded vigorously to air puffs directed at their cerci, showing that escape behaviour remained fully functional in the colony and had not somehow been lost by inbreeding.

Attempts to develop conditioned responses to 1.7 kHz tone pulses of moderate intensity which alone evoked no response, also failed. Cockroaches recently removed from the colony were restrained on the platform apparatus shown in Fig. 13. For three individuals tested, no detectable association was formed between the tone and the response to a paired electric shock, although each shock reliably elicited twitches from

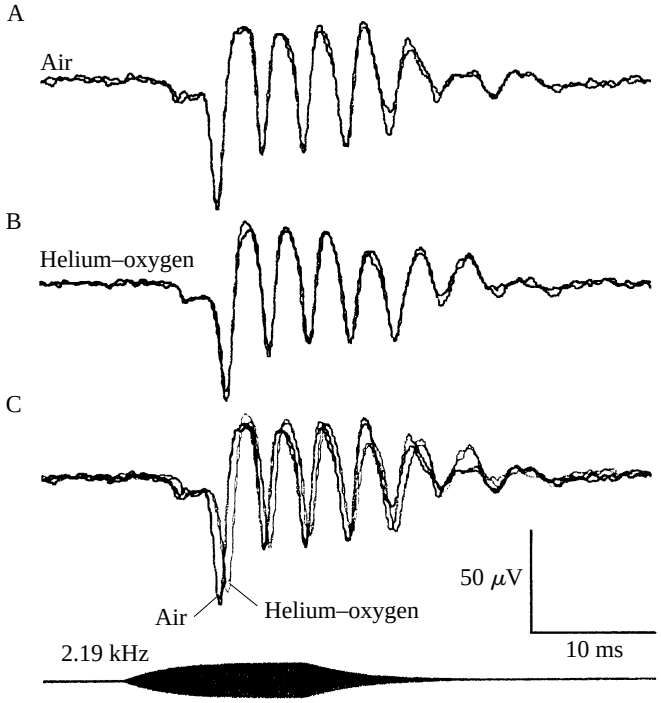

Fig. 12. (A) Two averaged responses to sound (*N*=256 each) recorded from a metathoracic leg suspended in air, obtained in alternation with (B) two averages from the same leg at the same position in a local atmosphere of helium–oxygen, in which the speed of sound is 1.85-fold higher. There is no significant change in response oscillation frequency in $He–O_2$, but a small overall phase lag is evident when the same two sets of recordings are superimposed, in C.

the legs, and a range of intervals separating the two stimuli was tried. In two further tests, no associations were formed between a flash from a green light-emitting diode and the same shock response or between a sound and the natural escape response to an air puff directed at the cerci. Others have reported difficulties in conditioning cockroaches (Huber *et al.* 1990).

Using brief loud tones, clear responses were elicited reliably from both males and females on most presentations of the sound alone, in ten animals examined under dim, constant illumination (Fig. 13B). Responses had a short latency (approximately 25 ms) but typically moved the measuring platform less than 50 μm and generally could not be detected by eye. A larger response was sometimes evoked by the first presentation after a period of silence and was occasionally followed by struggling. The short-latency response subsequently habituated somewhat, but could be restored or even enhanced (sensitized) in two preparations tested by brushing the cockroach lightly, electrically shocking it or delivering air puffs to the cerci between sound presentations.

In these tests, a high frequency (usually 3 kHz) was used, with the speaker positioned 48 cm (about 4 wavelengths) posterio-lateral to the cockroach, to avoid low frequencies and near-field conditions that might stimulate the cerci, the hairs of which are particle

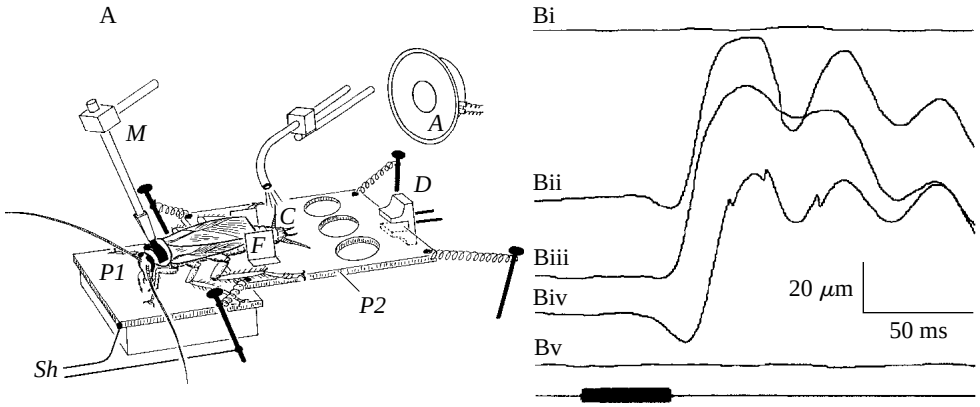

Fig. 13. Evidence for behavioural responsiveness to sound. (A) A micromanipulator *M* holds a yoke waxed to the protergite, so that the cockroach straddles a fixed metal platform, *P1*, and one that is lightly spring-loaded, *P2*. Any movement of *P2* is sensed by a position detector *D*. The auditory stimulus was a tone pulse from a speaker (*A*) placed 48 cm posteriolaterally, but the cockroach could also be stimulated or sensitized by shocking the tarsi using a pulse generator connected across the platforms (*Sh*) or by an air puff aimed at one cercus (*C*). Fins, *F*, restricted lateral abdominal movement. (B) Mechanical responses from a male cockroach signal-averaged from detector *D* (*N*=20 each trace) to loud (105 dB SPL) 3.00 kHz tones (lowest trace) presented 10 s apart. (i) No stimulus, cerci intact; (ii) stimulus tone present, cerci intact; (iii) stimulus present, cerci and anal styles coated with high vacuum grease; (iv) cerci excised and stumps re-coated with grease; (v) no stimulus again. The large oscillations in ii–iv arise from the lightly damped resonance of *P2*.

velocity detectors (Tautz, 1979; see Materials and methods). Cercal tuning in cockroaches seems only to have been measured in conditions where the calibrations would have been compromised by near-field effects (Counter, 1976; Schwab and Josephson, 1977), but is expected to be maximal below 100 Hz as found in *Henschoutedenia* and in some Orthoptera (Guthrie, 1966; Tautz, 1979). In five animals tested further, the response to a 3 kHz tone was not diminished by coating the cerci with silicone grease or petroleum jelly, which immobilizes the hairs, nor by excising both cerci as well (Fig. 13Biii,iv). While this clearly eliminates the cercal hairs as the source of the motor reaction to a 3 kHz tone, it is insufficient to implicate any of the subgenual organs for certain, since other sound receptors may remain to be discovered. The only other putative sound detectors that have been described are found in lateral folds of the abdomen and consist of external spines sensitive to air currents and to high-intensity near-field clicks (Florentine, 1967). Because these receptors are therefore almost certainly also low-frequency particle velocity detectors like the cercal hairs, they are also unlikely to be involved in mediating the motor responses to the high-frequency sound pressure changes just described.

Although these tests clearly establish for the first time that *P. americana* can hear sounds in the frequency band where the subgenual organs are most sensitive, most responses to sound were subthreshold for escape behaviour and could not be detected by eye. This explains the earlier failure to elicit obvious responses from individuals in the

colony, and suggests that sound inputs normally may be of functional importance in this species only when coupled with other inputs to enhance arousal (Ritzmann *et al.* 1991), or where a sound occurs after a long period of silence.

Discussion

Auditory sensitivity in a 'vibration' detector

The subgenual organs in the tibiae of the cockroach *Periplaneta americana* have long been known as the most sensitive vibration detectors among insects (Autrum and Schneider, 1948; Schnorbus, 1971; reviewed by Dambach, 1989). The results here suggest that they are, if anything, more sensitive to sound than to vibration, when judged by the energy required to elicit criterion responses through the two modalities. This observation alone makes it highly improbable that sound is detected as substratum vibration: an extremely low relative sensitivity to sound would then be anticipated because of the expected inefficient transformation of sound energy into substratum vibration.

The limited literature on the SGO comes close at several points to suggesting that the organ in cockroaches might have an auditory capability (Howse, 1964, 1968; Schnorbus, 1971; Moran and Rowley, 1975), but this general idea seems not to have been tested directly on the SGO outside the seminal work of Autrum, and others since, on tettigoniids (Autrum, 1941; Kalmring, 1985; Römer, 1985). After ablation experiments on all three pairs of legs in two tettigoniid species, Autrum (1941) concluded that airborne sound was detected by two tibial organs, the crista acoustica for high frequencies by direct pick-up and the SGO for frequencies below about 1 kHz. Because the SGO (but not the crista) in the same preparations was also extremely sensitive to vibration, Autrum could not decide whether the response in the lower frequency range was also direct, whether it was mediated by sound-induced substratum vibration, or whether by vibration of the leg itself, and he explicitly left these questions open. The suggestion that sound-induced substratum vibration is the link by which sound is detected has passed into the literature through repetition by subsequent commentators as if it were established fact (Howse, 1964; Wigglesworth, 1972; Moran and Rowley, 1975; Elsner and Popov, 1978), although the sound thresholds measured by Autrum (1941), at 45–50 dB SPL, actually were not high but lay within the range of subsequent determinations for well-validated insect auditory systems. There appears to be no direct evidence supporting the substratum-vibration hypothesis. The second idea of Autrum (1941), that an unspecialized leg can vibrate sufficiently in a sound field to cause the response (see also Schnorbus, 1971), also seemed unlikely from the outset, because the electrical response and its sensitivity remain largely unaffected by whether the leg is left hanging free or is firmly restrained by being waxed to supports, and when it is immersed in water. Critical refutation comes from experiments in which most of the leg is enclosed inside a small sound-proof chamber, with only the cut coxal surface exposed. Sensitivity to sound can actually be enhanced by this manipulation, showing that strong activation of the SGO can be obtained even in the complete absence of sound access to the surface of the leg (S. R. Shaw, D. S. MacIntosh and K. M. Kokic, in preparation). This second idea of Autrum's can be re-stated more

usefully in modern terms as a requirement for some form of efficient acoustic impedance-matching device (such as a vibrating tympanum) to transfer significant amounts of sound energy into the leg in a manner that will displace the sense organ (Larsen *et al.* 1989). This raises the question of the existence and location of a tympanal equivalent in the cockroach leg. Results obtained in connection with Fig. 10 suggest that points where tracheal air-sacs closely approach the surface cuticle may be prime candidates.

The cockroach tibia does not have an elaborate tympanal organ, only the SGO and two adjoining structures (the *Nebenorgan* and *Distalorgan*) each containing a few cells and apparently responding to low-frequency vibration (Schnorbus, 1971). This leaves the SGO as the main candidate for sound reception at frequencies around 2 kHz, with the long-latency response to sound frequencies near 250 Hz (Fig. 9B) perhaps attributable to the *Distalorgan* (the metathoracic *Nebenorgan* attaches to the wall of the leg, not to the tracheae). The sound responses described here do not come from the many external hairs on the leg, as shown by immobilizing these with petroleum jelly or water. Comparable results on tettigonids led Autrum (1941) to the same conclusion.

Evidence presented here helps resolve the original uncertainty of Autrum (1941) about the mechanism of sound detection. In *Periplaneta americana*, detection is not mediated by sound-induced vibration of the ground being transferred to the leg (Fig. 7) nor by artefactual passage of vibrations from the speaker system, but by direct airborne radiative pick-up of sound by some structure inside the leg. The sound pressure level needed to produce a threshold response in the best cases (41 dB SPL, Fig. 8C) is comparable with that of several *bone fide* insect ears, although some with elaborate tympana are more sensitive (e.g. Michelsen and Nocke, 1974; Elsner and Popov, 1978; Oldfield, 1985). The source of the wide variation in measured SGO threshold (Fig. 8C) is not known, but a variable orientation of the leg with respect to the speaker may have raised the measured threshold in some tests, because the auditory response is directional (Fig. 11). Michelsen (1971) reported a comparably broad range of thresholds in the locust (abdominal) ear, which correlated with the amount of fat deposited in the body.

Both the vibrational and the auditory properties appear to be mediated by the same sense cells localized in the SGO, as determined from the similarity in form and response frequency of the oscillatory VEP and AEP (Figs 5, 7), from ablation experiments on the SGO (Fig. 7) and particularly from the results of cross-modal adaptation (Figs 6, 7C). If the VEP and AEP responses were produced by different organs in the same complex or by different cells lying within one SGO, the effectiveness of cross-modal adaptation would be difficult to explain. Adaptation would then have to take place at a site common to both sets of organs or cells, prior to the VEP and AEP transduction loci and external to the organ, and would therefore have to be mechanical in origin. There is no known basis for efferent-controlled adaptation (Schnorbus, 1971; Moran and Rowley, 1975), and all efferent circuits must anyway be disabled in an isolated leg. Stimulus-induced, cell-autonomous active stiffening of the cells without external neural intervention is not eliminated as a possibility, but in the vertebrate auditory system where this is proposed (Hudspeth, 1985) it is the accessory hair cells that stiffen, allowing the inner hair cells to retain their responsiveness. There is no evidence for such a division of labour in the SGO, in which all 26 chordotonal cells occupy equivalent positions (Schnorbus, 1971; Moran

and Rowley, 1975). The most likely explanation is therefore that the same chordotonal sensilla mediate the responses to both sound and vibration, especially since it now appears that both stimuli produce pressure fluctuations within the tracheae (Fig. 10).

Is sound an adequate stimulus for the subgenual organ?

To determine whether the SGO system of the cockroach is best considered as a detector of sound or of ground vibration requires information on the relative availability of suitable natural stimuli (Markl, 1983). The SGO receptors obviously respond to both modalities, as must the animal, since an evoked response to a tone can be made to be indistinguishable from that to a carefully chosen vibrational input. Dual responsiveness has also been proposed for the SGO of tettigoniids (e.g. Kalmring, 1985; Römer, 1985).

One reason for suspecting the importance of an auditory function is that most natural substrata markedly attenuate higher-frequency contact vibrations. For plant stems (Keuper *et al.* 1985), soil (Dambach, 1989) and sand (Brownell, 1977), most of the energy is concentrated in a band below a few hundred hertz and, even in wet sediments, attenuation rises in proportion to frequency (Hamilton, 1987). An organ with a poor low-frequency response like the SGO, which is tuned near 2 kHz, is therefore unlikely to be an efficient vibration detector on many surfaces. It could be effective at close range, however (Brownell, 1977; Markl, 1983; Keuper *et al.* 1985), or on rigid surfaces that attenuate high frequencies less, provided that sufficient vibrational energy is available. Comparative measurements are needed to resolve this question.

A second reason for suspecting that auditory input may be functional under some conditions is that the threshold energy required to activate the SGO with sound is similar to, or even lower than, that for vibrational input, with threshold sound pressure levels comparable to those for accepted insect ears (Fig. 8C).

Third, indirect evidence suggests that the response of the SGO to sound is not just an epiphenomenon. Comparing auditory threshold tests for units in the ventral cord of intact *Periplaneta* (Fig. 4 of Decker *et al.* 1989) with those of Schwab and Josephson (1977), who removed the legs, reveals similar responses at low frequency where cercal inputs would dominate, but enhanced sensitivity above 800 Hz in animals with their legs intact. A non-cercal auditory response, presumably from the SGO system, thus penetrates at least some distance into the nervous system. Some thoracic interneurones are responsive to airborne sound *via* inputs in the thoracic region, including the legs (Ritzmann *et al.* 1991).

Fourth, measurements of motor output (Fig. 13) confirm that cockroaches do respond to airborne tones in the range tested (1.3–5 kHz). Sound may occasionally trigger large responses, but most motor activity was barely detectable by eye and was below threshold for escape behaviour, again corroborating the findings of Ritzmann *et al.* (1991) on interneurones. There is no clear behavioural context in which to place the SGO response to sound, which is almost certainly not used in signalling in this species. Apart from low frequencies generated by wing fluttering (cf. Dambach, 1989), *P. americana* probably uses neither audible nor ultrasonic sounds for intraspecific communication (Gold *et al.* 1984) and appears to be a largely silent species, in common with other Blattidae. By contrast, members of the blaberid families of cockroach emit a variety of acoustic signals

through stridulation, wing-scraping or whirring, spiracular hissing or drumming that are presumed to be behaviourally significant (Roth and Hartman, 1967), and which, in one case, was sufficient to frighten off rodents (Guthrie, 1966). The weak behavioural responses to sound in *P. americana* could be vestiges of a condition that was more pronounced in an ancestral group and which has largely disappeared in this particular family (Roth and Hartman, 1967). Alternatively, a more appropriate behavioural context may remain to be discovered, such as flight. Auditory organs in several insect groups facilitate predator detection during flight, and this may have been their ancestral function before some groups developed acoustic communication (Hoy *et al.* 1989). Auditory responsiveness dependent upon the behavioural context has been reported recently in thoracic interneurones that activate motor neurones responsible for escape turning. Auditory signals combine with other inputs to form part of an arousal system that is driven multimodally, not just from the cercal wind-detectors, and a stimulus in one modality is less effective when delivered in isolation from other inputs (Ritzmann *et al.* 1991). The effectiveness of sound in the present case could be enhanced (sensitized) by other inputs, as reported generally by Zilber-Gachelin and Paupardin (1974). In locusts, Hoyle (1964) claimed that almost any arousing stimulus produced subthreshold excitation of many functionally unrelated muscles.

The origin of synchronized nerve impulses

A damped oscillatory response of nerve impulses synchronized at about 300 Hz (Fig. 4) has not been reported before from subgenual nerve recordings, probably because the only study that was technically adequate to reveal it (Schnorbus, 1971) was made without signal-averaging and used a continuous vibratory stimulus, during which the oscillatory response would have adapted and desynchronized (Fig. 1). The normal response recorded across the coxa–femur joint is thought to be a compound response in which the synchronized, conducted activity of many of the SGO axons in the nerve predominates, for instance because much of it can be blocked by cooling the axons selectively while the SGO itself remains at room temperature (Fig. 2). The form of the 300 Hz response suggests a damped resonance, because in any one preparation its periodicity remained invariant over the dynamic range investigated (Fig. 3B) and across successive response cycles (Figs 1–3). It is important to pursue the origins of this frequency invariance and of synchrony among axons because this should throw light on the nature of mechanical coupling to the transduction process in the SGO and related auditory organs, which has remained elusive (Larsen *et al.* 1989). Four possible mechanisms for synchronization and frequency invariance are obvious: strong electrical coupling between the sense cells, resonance of air in the tracheal expansion coupled to the organ, intrinsic electrical resonance synchronized in all the mechanoreceptors in the SGO, and mechanical resonance of the organ in its fluid environment.

In the first case, the receptor cell bodies and dendrites are non-contiguous, but because the axons have neither been identified in leg nerve 5 nor studied, electrical coupling more proximally cannot be excluded. Although axonal coupling might account for synchronization of impulses, it is not a complete hypothesis by itself, leaving unexplained the frequency invariance with intensity and time (Figs 1–3).

Second, the oscillatory response might be a product of selective tuning and vibration within the tracheal system at approximately 300 Hz, which the SGO passively follows. This would obviously not be a direct resonance at the best stimulus frequency for vibration (Autrum and Schneider, 1948; Schnorbus, 1971) or for airborne sound (Fig. 8A,B), which are both centred at about 2 kHz, but might be a Helmholtz resonance (Morse, 1948) induced at a much lower frequency by a gourd-like expansion of the main trachea at the site of the SGO. This hypothesis predicts that the resonance fundamental frequency (f_R) should vary in proportion to the speed of sound c in the medium inside the tracheae. It can therefore be decisively rejected, because no detectable change in f_R follows replacement of air in the tracheae by helium–oxygen in which c is 1.85-fold higher (Fig. 12).

Third, the 300 Hz oscillation could represent tuned electrical resonances in the SGO sensilla, as found in reptilian hair cells (Fettiplace, 1987). A form of cell-autonomous tuning is also claimed for cricket auditory receptors (Oldfield, 1985, 1988). For the SGO, this hypothesis faces the difficulty that the extended slope of the response–dB curve of nerve impulse discharges (Fig. 3B) may imply that the sensilla of one SGO have different dynamic ranges, and so reach threshold at different intensity levels or at different times after the start of a stimulus. There would then be nothing to compel axonal synchronization, in contrast to observations at all but the lowest intensity levels (Fig. 3A). Alternatively, if all 20 or so sensilla possessed identical mean thresholds, the slope of the amplitude–dB curve would have to be interpreted as reflecting an increasing aggregate probability of impulses arising across the ensemble, as intensity rises. This and the observed response fluctuation (Fig. 3D) could be explained, in turn, only if the sensilla experienced substantial random variations of their individual thresholds over time, equivalent to adding some source of internal noise. The sensilla that became active would be those in which the time-varying threshold was sufficiently low at that moment to be triggered by the stimulus. The degree of synchrony achieved would depend on whether the activation process was fast relative to the time structure of the intrinsic noise and on the degree to which individual cells receive in-phase mechanical input. If noise amplitude were relatively independent of sound intensity, greater synchrony would be predicted as activation became larger. An estimator of this synchronization, quality factor Q, should rise with increasing intensity, as is observed (Fig. 3C). Response frequency would be set by the cells having identical electrically tuned properties, whereas initial synchronization would depend upon them receiving a common mechanical input. The electrical resonator model therefore needs additional mechanical coordination.

The fourth idea of intrinsic mechanical resonance in the SGO is simpler and seems equally plausible at present. Synchrony of impulses and invariance of f_R with intensity and time may be explained as responses of the sensilla to a common periodic driving force. As above, some source of internal noise is needed to explain all of the results of Fig. 3. Because the 300 Hz oscillation occurs at a frequency several-fold lower than the vibration tuning optimum, vibrational stimuli could not act by displacing the SGO in a simple one-to-one manner as Howse (1964) supposed, and the response would therefore represent an intrinsic resonance of the organ and its local environment. Howse (1964, 1967) anticipated the idea of a local mechanical resonance from tests with a mechanical

model, but extracted no evidence from the actual SGO. The idea that the SGO might resonate as a unit is compatible with its structure: it is supported on a common basal membrane, and the 20 or so microtubule-containing attachment cells at its edge are mechanically linked to each neighbour by elaborate desmosomal junctions (S. R. Shaw, D. S. MacIntosh and K. M. Kokic, in preparation). This implies that whatever movement occurs at one transduction site must be strongly correlated across the group, perhaps even synchronized. Distinction between the hypotheses of communal mechanical resonance *versus* cell-autonomous electrical resonance preceded by a common mechanical impulse may be possible through single-unit recordings, currently in progress.

In either case, the similarity of the sound and vibrational tuning curves and of the energy requirements for each modality appears paradoxical unless both contact vibration and sound act similarly, but could be reconciled if both directly energize the air in the tracheal system. This is contrary to earlier ideas that such vibrations are conducted exclusively in the haemolymph (Autrum, 1941; Schnorbus, 1971) but is supported by the observation that deforming the leg generates pressure transients within the tracheal system (Fig. 10).

Evolutionary implications

Originally, vibrational stimuli would have reached the SGO not by deforming the tracheae as just suggested, but by transmission through the haemolymph as usually supposed. A largely unrecognized problem for such a route is that, in the absence of some compressible element in the leg, the pressure component of transmitted vibrations in an incompressible haemolymph surrounded by a solid tibial cuticle up to 50 μm thick would have little effect in displacing the SGO structure differentially: SGO cells composed mainly of water would be nearly as transparent to the pressure change as the surrounding fluid, resulting in a transducer that is relatively inefficient for this component (Moran and Rowley, 1975; Schuijf and Buwalda, 1981). The SGO of one termite species is apparently disposed to act in this way (Fig. 14A) and is about 100-fold less sensitive to displacement than that of the cockroach (Howse, 1964, 1968). In this context, the functional significance of the large tibial tracheal expansion at the site of the SGO might have been the provision of a compressible air-sac to which one edge of the SGO attached, the rest being anchored to the immovable cuticle: expansion and compression of the tracheal sac during vibrationally induced pressure waves could then serve to amplify displacement of the SGO by the pressure component (Fig. 14B). An extra mechanotransducing advantage would then ensue, if mechanical deformation of the leg were able, in addition, to distort the tracheae, generating internal ‘sound’. Some previous studies incidentally illustrate a linkage from the SGO to the tracheae by strands of connective tissue (Howse, 1965, 1967, 1968; Schnorbus, 1971), but seem not to have found this noteworthy. In fact, this linkage must be of major significance for the functioning of the organ: we find that it is one of the main supports suspending the basal membrane that carries the entire SGO, the other being anchor points on the tibial wall opposite (Fig. 14B; Shaw *et al.* 1992; S. R. Shaw, K. M. Kokic and D. S. MacIntosh, in preparation). A seeming impediment to this proposed evolution is the need to generate a tracheal expansion, but these are very common in both major subgroups of extant cockroaches (Baudet and Sellier, 1975), so must have evolved

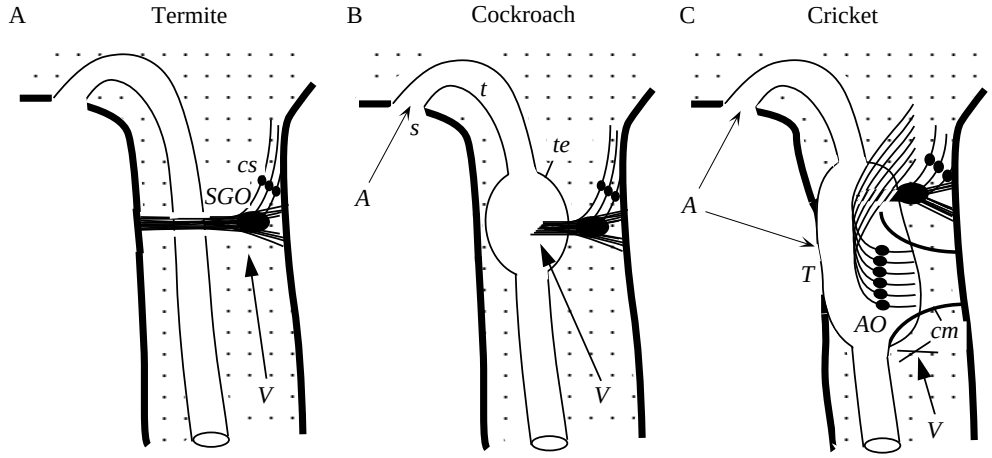

Fig. 14. Schematic diagram of three major steps (A–C) envisaged in the evolutionary transformation of a vibration-sensitive subgenual organ (*SGO*) into an auditory organ (*AO*). (A) The precursor state still found in some extant termites. The SGO with its inserted chordotonal sensilla (*cs*) is suspended in haemolymph between opposite walls of the tibia, creating a relatively insensitive detector of vibrations (*V*) transmitted in the haemolymph. (B) Sensitivity to vibration can be increased by coupling one edge of the SGO to a compressible tracheal expansion (*te*), as is found in cockroach tibiae. This pre-adapts the SGO to detect auditory stimuli (*A*) that enter the leg's trachea (*t*) *via* spiracles (*s*) on the body wall, producing a bimodal, combined vibration-and-sound detector. (C) In some crickets, a more specialized auditory organ is elaborated from the distal edge of the SGO. Development of one or more tympana (*T*) further facilitates sound entry, and enclosure of the organ by a covering membrane (*cm*) may contribute to its vibration isolation.

early. Four such bladder complexes occur in the metathoracic leg alone, so coupling a sense organ to one of them might simply have involved capitalizing upon an existing structure.

This proposal that the original function of the SGO's tracheal expansion was to help detect the pressure component of vibration in a fluid thus parallels that envisaged for the much larger swimbladders of many fish. These are closed, gas-filled pressure-to-displacement transformers that capture water-borne vibrations in the sub-kilohertz range (Tavolga, 1971; Schuijf and Buwalda, 1981). By contrast, the tracheal passages in the cockroach connect to the air outside through the spiracles. Evolution of enhanced sensitivity to vibration would thus have preadapted the SGO to detect airborne sound entering the tracheal system, even in *Periplaneta* which has no obvious tympanum to facilitate sound energy transfer directly into the leg (Fig. 14B), neatly explaining how a bimodal organ could evolve from a unimodal contact vibration detector. The homologous organ in Ensifera has also been viewed as a preadaptation for audition, but by arguing from a developmental perspective (Meier and Reichert, 1990).

The more specialized type of auditory organ of Ensifera (Fig. 14C) apparently evolved as the distal elaboration of an existing subgenual organ (Meier and Reichert, 1990), the latter hitherto regarded primarily as a vibration detector. The results here from cockroaches (a group that has retained many primitive features) suggest that the SGO in

the lower Carboniferous common ancestor of cockroaches and crickets may well already have possessed dual responsiveness to vibration and sound. The crucial new feature in the evolution of cricket hearing organs would have been development of their effective isolation from vibrations in the haemolymph, to account for the original observations of Autrum (1941) that the crista in tettigonids is insensitive to contact vibration. This requirement for isolation seems not to have been identified as a problem in the literature, but the crista lies next to the SGO in the same haemolymph channel (Eibl, 1978). Its vibration-isolating mechanisms are unknown, but might be one function of the expanded tracheae that fill the leg at this point, almost excluding the haemolymph, and of the tent-like structure that encloses the auditory organ in several crickets (Fig. 14C; Ball *et al.* 1989). Likewise, having intrinsically tuned sensilla (Oldfield, 1985) should effectively filter out low-frequency vibrations transmitted in the leg. Conversely, development of a tympanum (Fig. 14C) selectively enhances acoustic input at higher frequencies in some cases (Elsner and Popov, 1978).

It remains to be seen how *Periplaneta* achieves respectably low sound thresholds (Fig. 8C) rivalling several accepted insect ears, even in the absence of a differentiated tympanum that is usually associated with high sensitivity (Bennet-Clark, 1984; Larsen *et al*. 1989). In lacking a distinct tympanum, the cockroach SGO resembles the tibial auditory organ of a silent cricket, *Phaeophilacris spectrum* (Ball *et al.* 1989), suggesting that our current investigation of the manner of sound entry into a cockroach leg might illuminate the evolution of hearing even in some Ensifera.

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