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TYMPANIC MEMBRANE OSCILLATIONS AND AUDITORY RECEPTOR ACTIVITY IN THE STRIDULATING CRICKET *GRYLLUS BIMACULATUS*

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Summary

The ears of stridulating crickets are exposed to loud self-generated sounds that might desensitise the auditory system and reduce its responsiveness to environmental sounds. We examined whether crickets prevent self-induced auditory desensitisation, and measured the responsiveness of the peripheral auditory system of the cricket (acoustic spiracle, tympanic membrane and tympanic nerve) during pharmacologically induced sonorous (two-winged) and silent (one-winged) stridulation.

The acoustic spiracles remained open during stridulation, so the self-generated auditory signal had full access to both the external side and the internal side of the tympanic membrane. When the spiracles shut in resting crickets, the responsiveness of the tympanic membrane to acoustic stimuli varied according to the phase of ventilation and was minimal during expiration. The tympanic membrane oscillated in phase with the self-generated sounds during sonorous chirps and did not oscillate during silent chirps. In both sonorously and silently singing crickets, the responses of the tympanic membrane to acoustic stimuli were identical during the chirps and the chirp intervals.

Bursts of activity were recorded in the tympanic nerve during sonorous chirps; however, activity was minor during silent chirps. In sonorously and in silently singing crickets, the summed nerve response to acoustic stimuli in the chirp intervals was the same as in resting crickets. The response to stimuli presented during the syllable intervals of sonorous chirps was slightly reduced compared with the response in the chirp intervals as a consequence of receptor habituation. In silently singing crickets, acoustic stimuli elicited the same summed nerve response during chirps and chirp intervals. These data indicate that in the cricket no specific mechanism acts to reduce the responsiveness of the peripheral auditory pathway during stridulation.

Key words: *Gryllus bimaculatus*, cricket, stridulation, hearing, laser interferometry, laser vibrometry, tympanic membrane, tympanic nerve, acoustic spiracle.

Introduction

A fundamental problem of sensory processing is the discrimination between self-generated, or reafferent, sensory information and externally evoked sensory information of the same modality. This is especially important in communicating animals that broadcast high-intensity signals that could desensitise their own sensory pathways for some time. A solution common to many sensory systems is to reduce the responsiveness of the sensory pathway during the generation of reafferent information. Modulation of the responses to reafferent sensory information can occur at two stages of the sensory pathway: peripherally, as a result of mechanical changes at the sense organ, or centrally within the nervous system. In auditory systems, peripheral modulation of the hearing organ may result from a change in its biophysical properties (Suga and Jen, 1975; Borg and Counter, 1989; Hennig et al., 1994; Narins, 1992). For example, in vertebrates, the stapedius and tensor tympani muscles contract during sound production, which dampens self-generated vibrations of the ossicles of the ears (Borg and Counter, 1989). The auditory threshold of the cicada is increased by 20 dB SPL during sound production as a result of folding of the tympanic membranes (Hennig et al., 1994). Central neuronal mechanisms that reduce the response of auditory neurons to self-generated sound have been identified in the bat (Suga and Schlegel, 1972; Suga and Shimozawa, 1974; Schuller, 1979; Metzner, 1993), monkey (Müller-Preuss and Ploog, 1981) and human (Paus et al., 1996).

Discrimination between self-generated and environmental sounds is a problem in stridulating crickets. Stridulating male *Gryllus bimaculatus* generate loud (102 dB SPL at a distance of 50 mm: Nocke, 1972), repetitive chirps by rubbing their forewings together rhythmically. Exposure to sound stimuli causes habituation of auditory afferents (Esch et al., 1980; Ocker and Hedwig, 1993; Givois and Pollack, 2000) and elicits an inhibition of cricket auditory interneurons, with a time course dependent on the duration and intensity of the sound

(Pollack, 1988). This suggests that loud self-generated sounds should impede the ability of a cricket to hear subsequent environmental sounds. However, stridulating male crickets show behavioural responses to sound presented externally: they will alter their chirp rate if presented with an acoustic stimulus in the chirp interval (Heiligenberg, 1969; Jones and Dambach, 1973). How does a cricket maintain auditory responsiveness during stridulation despite the massive self-generated auditory stimulation? To answer this question, we analysed the responses of the peripheral and central auditory pathways of the cricket during stridulation induced by injection of pharmacological agents into the brain (Otto, 1978; Wenzel and Hedwig, 1999). In this paper, we report on peripheral sound processing during stridulation.

Materials and methods

Preparation of animals and eliciting stridulation

All experiments were performed at room temperature (18–22 °C) using adult *Gryllus bimaculatus* DeGeer. Singing males, with intact auditory organs and wings, were selected from our colony, which is maintained on a 12 h:12 h light:dark cycle at 25 °C. Prior to dissection, animals were kept at 4 °C for approximately 45 min. The crickets were restrained in a standing posture on a Plasticene-covered platform. Metal hooks were used to secure all the legs to the platform. The head was waxed to a moveable metal support, and the frontal region of the head cuticle was removed to expose the brain for stimulation. Stridulation was initiated by injection of the acetylcholine esterase inhibitor eserine (10^{-2} mol l^{-1}) into the frontal protocerebrum (Otto, 1978; Wenzel and Hedwig, 1999). We examined two-winged sonorously stridulating crickets or, after removal of their right wing, one-winged silently stridulating crickets. All exposed nervous tissue was bathed in insect saline (ionic composition, mmol l^{-1}: NaCl, 140; KCl, 10; $CaCl_2$, 4; $NaHCO_3$, 4; $NaHPO_4$, 6).

Laser measurements

Twenty-one crickets were used for laser measurements. Experiments were performed on a 4000 kg steel platform that had been set in concrete to isolate it from surrounding floor vibrations. We used a laser vibrometer to measure tympanic membrane oscillations and a laser interferometer to measure displacements (Polytech OFV 3000 controller with a Polytech OFV 302 H sensor head). The laser beam was focused onto a reflective glass bead (diameter 70 μm, mass 0.2 μg) glued to the tympanic membrane. The laser vibrometer calculated the Doppler shift between the reflected and the reference beam to determine the velocity of tympanic membrane oscillations. The full frequency range for the vibrometer was 1 Hz to 150 kHz; however, we used a low-pass filter with a cut-off frequency of 20 kHz. An integrated circuit (Analog Devices; type 637 JD), with an integration time of 1 ms, then computed online the root mean square (RMS) of the velocity signal. The laser interferometer compared the phase shift between the reflected beam and the reference beam to produce the amplitude of slow displacements of the tympanic membrane (frequency, direct current to 50 kHz). The full displacement range of the interferometer was set to ±640 μm with a resolution of 0.32 μm.

Recording of tympanic nerve activity

Extracellular recordings of the tympanic nerve of 15 crickets were made at rest and during stridulation. The axons of approximately 60 auditory afferent neurons are all contained in the dorsal branch of prothoracic nerve 5 (the tympanic nerve) in the femur of the foreleg (Michel, 1974). This branch also contains the axons of some other mechanosensory neurons from the subgenual organ and some campaniform sensilla. To stabilise the recordings, the forelegs were waxed to two thin steel wires. The tympanic nerve was then exposed by removing a rectangle of cuticle from the dorsal part of the femur. The silver wire indifferent electrode was placed in contact with the haemolymph distal to the recording site, and the silver wire recording electrode was hooked underneath the nerve and gently raised above the haemolymph. Once a stable recording had been obtained, the recording electrode and nerve were insulated with Vaseline.

Acoustic stimulation and recording

Acoustic stimuli were presented from two piezo-electric speakers through brass tubes with a diameter of 14 mm, positioned 13 cm from the posterior tympanic membrane. The acoustic stimuli were generated using Cool Edit 1998 software (Syntrillium) running on a Toshiba laptop (CD 4010). We presented short (8 ms) sound pulses with an interpulse interval of 7 ms at the calling song frequency of the cricket (4.5 kHz). All stimuli were 90 dB SPL re 20 μPa in amplitude. Sound pulses were calibrated beforehand with a measurement amplifier (Brüel & Kjær: type 2610). During the experiment, a microphone (Audio-Technica AT853A), positioned 5 cm from the forewings, recorded acoustic stimuli and sound produced by the cricket. Because of the directionality of the microphone, recordings of sound stimuli are of relatively small amplitude compared with recordings of the sound generated by the cricket. The microphone recordings were therefore used only as qualitative references for sound production; they were not used to calculate absolute sound amplitude. Note that, in figures of high temporal resolution, the recordings of acoustic signals (4.5 kHz) appear to be discontinuous; this is a result of sampling the data at 10 kHz.

Recording of behaviour

Movements of the cricket were recorded using either a high-speed video camera (Redlake Imaging PCI 2000 S) or optoelectronic cameras (Hedwig, 2000a). The optoelectronic cameras were focused onto a reflective disk (diameter 2 mm; 3M Scotchlite 7610) glued to the relevant body part. In this way, we recorded stridulatory wing movements together with either ventilatory abdominal pumping movements or acoustic spiracle opening and closing. When recording spiracle movement, a smaller reflective disk (diameter 0.5 mm) was

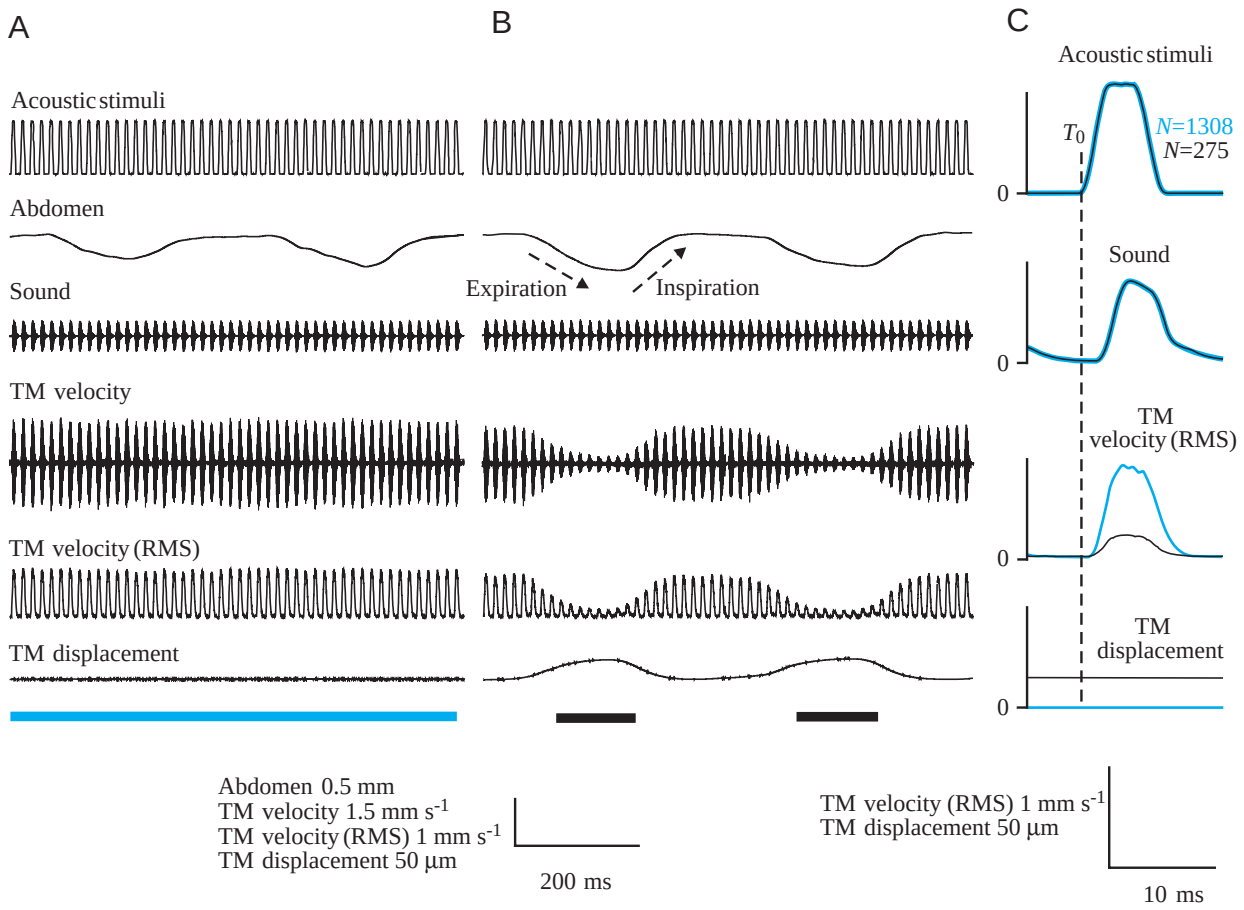

Fig. 1. Responses of the tympanic membrane in the resting, ventilating cricket. (A) Constant response of the tympanic membrane during ventilation. (B) Decrease in the amplitude of the tympanic membrane oscillations in synchrony with ventilation in the same cricket. (C) Averaged recordings of acoustic stimuli, sound pattern, tympanic membrane velocity (root mean square, RMS) and tympanic membrane displacement while at rest (in blue, made from the recordings above the blue bar in A) and during the phase of decreased responsiveness (in black, made from the recording above the black bars in B). In C, T_0 signifies the trigger point for the averaging process, and the blue traces have been made twice as thick as the black traces to aid discrimination. Acoustic stimuli, 8 ms, 4.5 kHz, 90 dB SPL; TM velocity, velocity of the tympanic membrane oscillations; TM velocity (RMS), the root mean square of the velocity of the tympanic membrane oscillations; TM displacement, displacement of the tympanic membrane; Abdomen, movement of the abdomen; Sound, sound recordings.

glued to the large dorsal spiracular flap, while the pronotum and cuticle surrounding the spiracle were waxed to a supporting pin embedded in the Plasticene-covered platform. This isolated the movement of the spiracle from any body movements.

Data sampling

All recordings were sampled online to the hard disk of a computer *via* a high-speed A/D board (DT 2821 F8D1) run under Turbolab 4.0, and later stored on compact disc. The sampling rate was 10 kHz per channel. Data were subsequently analysed using the software Neurolab (Knepper and Hedwig, 1997). Data presented in the figures are representative examples from all the animals analysed. The responses to acoustic sound pulses were separated prior to analysis depending on whether they had been presented during chirps or during chirp intervals. Extracellular nerve recordings and the microphone recording were full-wave-rectified prior to averaging. When averaging data, the start of either the acoustic stimuli or wing movement was used as the temporal reference point (T_0).

Results

The peripheral auditory system

The primary sound receivers of the peripheral auditory system of the cricket are the posterior tympanic membranes, located on the tibiae of the forelegs (for reviews of sound reception, see Larsen et al., 1989; Michelsen, 1998). Both posterior tympanic membranes are connected to each other and to the acoustic spiracles on the thorax by an H-shaped tracheal system, the acoustic trachea. Oscillations of the posterior tympanic membranes are sufficient to generate auditory responses in the nervous system (Kleindienst et al., 1983). The magnitude of the oscillations is determined by both externally and internally transmitted sound (Hill and Boyan, 1976; Larsen

and Michelsen, 1978; Michelsen et al., 1994). One route that internally transmitted sound takes is *via* the acoustic spiracles. An analysis of peripheral auditory responsiveness should therefore consider the opening and closing movements of the auditory spiracles.

The acoustic spiracles

In the first series of experiments, we presented acoustic stimuli to the cricket and made laser vibrometer/interferometer measurements of tympanic membrane oscillations and displacement in otherwise resting crickets (Fig. 1A). Normally, there were no displacements of the tympanic membrane caused by ventilation, and it oscillated regularly in response to the acoustic pulses with a maximum RMS velocity of 0.9 mm s^{-1} (blue trace in Fig. 1C). In one animal, however, we recorded 20 µm outward displacements of the tympanic membrane in synchrony with abdominal pumping movements (Fig. 1B). These movements were accompanied by a decrease in the amplitude of the sound-induced membrane oscillations. At the peak of the slow displacements, the mean amplitude of the tympanic membrane oscillations was reduced to a maximum RMS velocity of 0.21 mm s^{-1} (black trace in Fig. 1C), which is equivalent to a 12.6 dB decrease in sensitivity. We were able to mimic this effect in 10 animals by waxing their spiracles shut (data not shown). Closing the acoustic spiracles could, therefore, be an effective way of controlling the amplitude of reafferent auditory input.

We recorded the opening and closing movements of the left acoustic spiracle with a high-speed video camera and an optoelectronic camera. While the crickets were resting, the acoustic spiracle was normally in the open state (Fig. 2A). When we touched the animal briefly, the acoustic spiracle closed and immediately opened again (Fig. 2A), indicating that crickets have control over the opening state of their acoustic spiracle. When the crickets stridulated, however, the spiracle remained open (Fig. 2B). Thus, there is no evidence that the cricket *Gryllus bimaculatus* uses its spiracles to control input to its auditory pathway during stridulation.

Tympanic membrane oscillations and displacement during stridulation

We next recorded tympanic membrane oscillations and displacements during stridulation to determine whether the tympanic membrane responds to self-generated sounds. The tympanic membrane oscillations recorded mirrored both the amplitude and the timing of the stridulatory sound pattern picked up by the microphone (Fig. 3A). There were no low-frequency displacements of the tympanic membrane in the seven sonorously or the six silently singing crickets that we recorded (Figs 3, 4). The maximum RMS velocity during stridulation was 11 mm s^{-1} (Fig. 3A), but since sound was not produced with the same intensity in every syllable, the maximum of the averaged RMS signal was only 3.4 mm s^{-1} (Fig. 3B). We did not record any oscillations of the tympanic membrane during silent singing (Fig. 3C,D). This confirmed that the tympanic membrane of the cricket was oscillating in response to sound production, rather than in response to motor activity during wing movement alone.

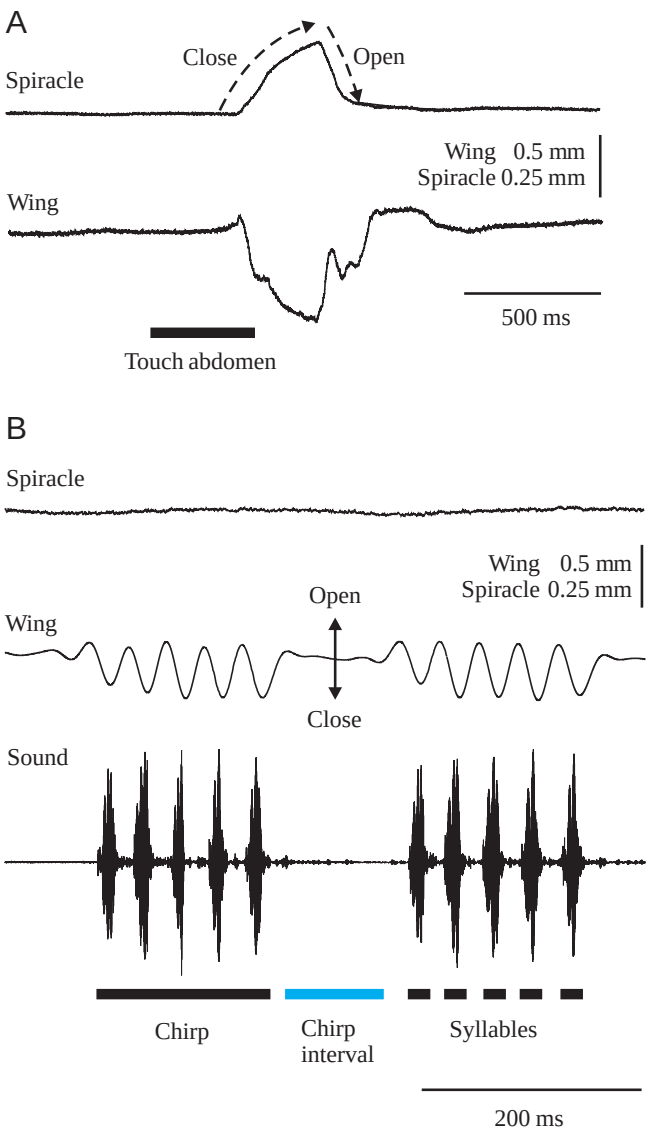

Fig. 2. (A) The acoustic spiracle was open when the cricket was at rest but closed briefly when the cricket was touched on the abdomen with a paintbrush. (B) The acoustic spiracle remained open during stridulation. A chirp, chirp interval and the syllables that make up a chirp have been marked below the sound recording. Spiracle, movement of the acoustic spiracle; Wing, stridulatory wing movements.

Responsiveness of the tympanic membrane to acoustic stimulation during stridulation

Although the tympanic membrane oscillated in response to the sound generated by the cricket, the magnitude of the oscillations could still be a target of modulation. We therefore presented calibrated sound pulses of 90 dB SPL during stridulation and evaluated the amplitude of the tympanic membrane oscillations. Responses during the chirp intervals were identical to responses in a resting cricket (data not

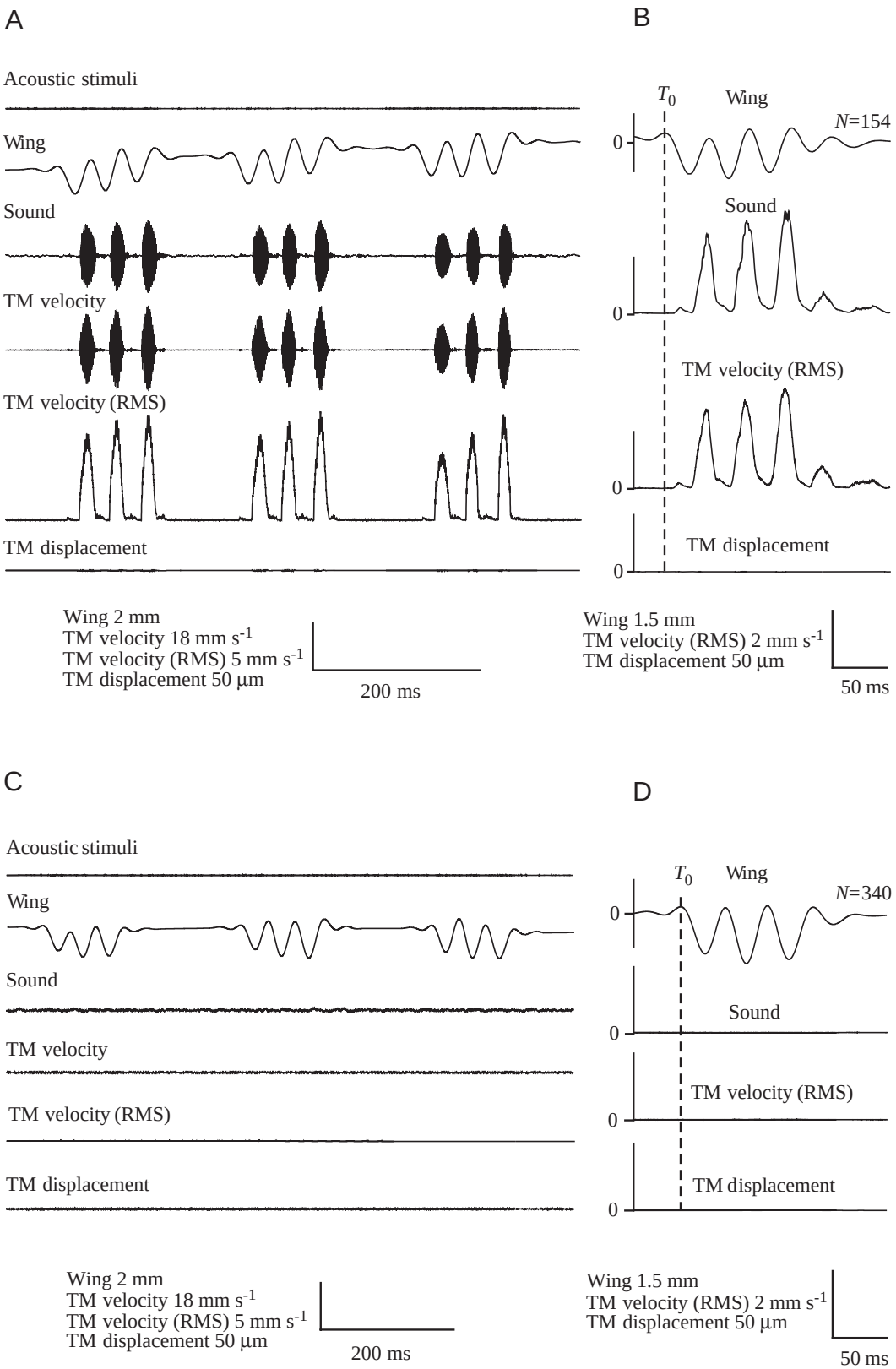

Fig. 3. Tympanic membrane oscillations during stridulation. (A) During sonorous chirps, the tympanic membrane oscillated vigorously but did not show any low-frequency displacement. (B) Average wing movement, sound pattern and tympanic membrane oscillations during 154 sonorous chirps. (C) During silent stridulation, the tympanic membrane did not oscillate or show any low-frequency displacement. (D) Average wing movement, sound pattern and tympanic membrane oscillations during 340 silent chirps. Wing, stridulatory wing movements. For further details, see Fig. 1.

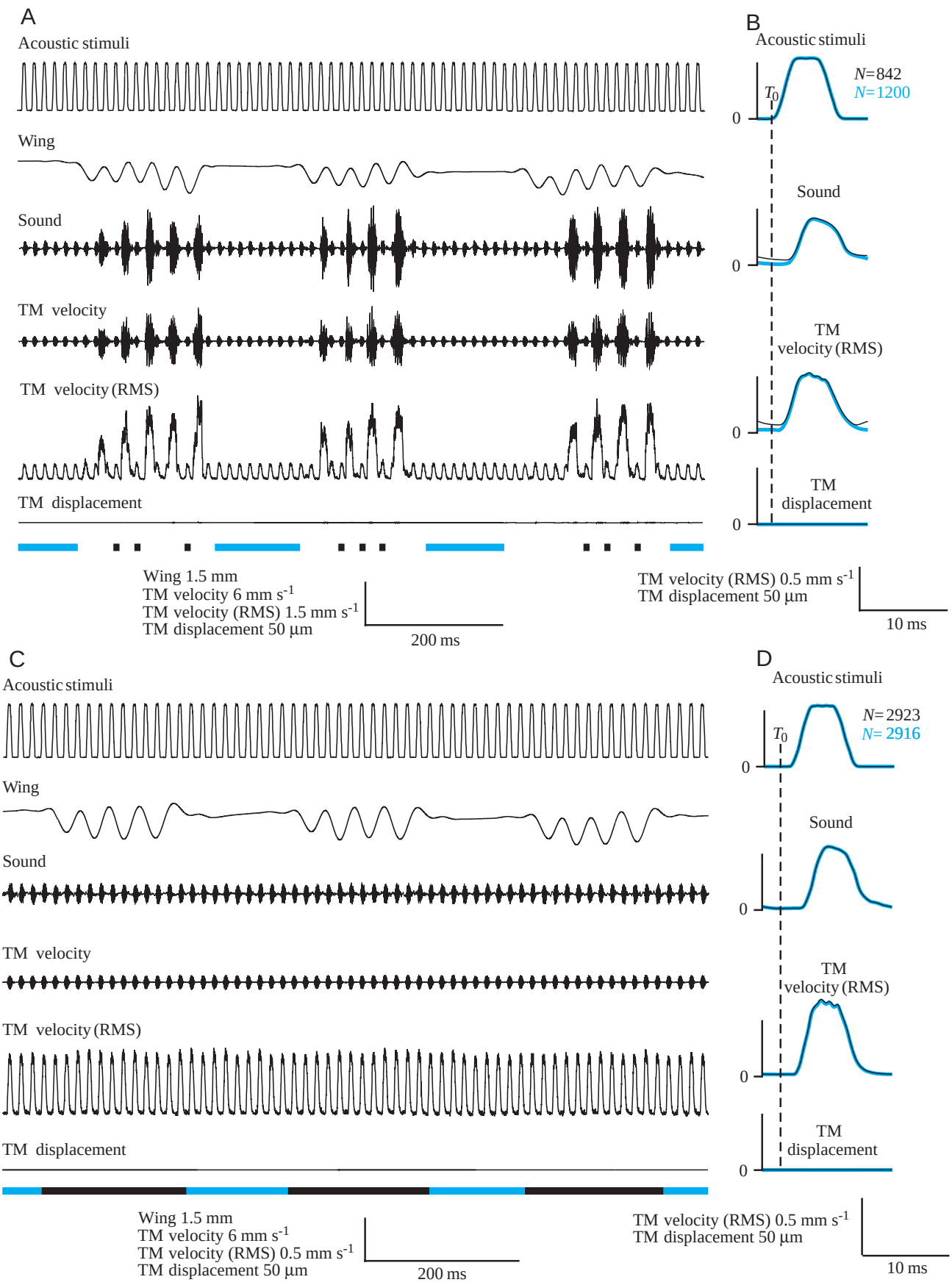
A
Acoustic stimuli
Wing
Sound
TM velocity
TM velocity (RMS)
TM displacement
B
Acoustic stimuli
N=842
N=1200
T0
0
Sound
TM velocity (RMS)
TM displacement
Wing 1.5 mm
TM velocity 6 mm s-1
TM velocity (RMS) 1.5 mm s-1
TM displacement 50 µm
200 ms
TM velocity (RMS) 0.5 mm s-1
TM displacement 50 µm
10 ms
C
Acoustic stimuli
Wing
Sound
TM velocity
TM velocity (RMS)
TM displacement
D
Acoustic stimuli
N=2923
N=2916
Wing 1.5 mm
TM velocity 6 mm s-1
TM velocity (RMS) 0.5 mm s-1
TM displacement 50 µm
200 ms
TM velocity (RMS) 0.5 mm s-1
TM displacement 50 µm
10 ms

Fig. 4

Fig. 4. Tympanic membrane oscillations in response to acoustic sound pulses presented during stridulation. (A) The tympanic membrane oscillated in response to the acoustic stimuli with the same root mean square (RMS) amplitude during the chirp intervals (marked by blue bars) as during the intersyllable intervals of chirps (marked by black bars). (B) Overlaid averages of the tympanic membrane oscillations (RMS) in response to sound patterns generated by acoustic stimuli presented during intersyllable intervals (black) and chirp intervals (blue). (C) During silent stridulation, the RMS amplitude of the tympanic membrane oscillations remained the same during chirps (black bars) and chirp intervals (blue bars). (D) Overlaid averages of the tympanic membrane oscillations (RMS) in response to sound patterns generated by acoustic stimuli presented during silent chirps (black) and chirp intervals (blue). Wing, stridulatory wing movements. For further details, see Fig. 1.

shown). We compared the responses during the chirp intervals (blue bars) with the responses during the intersyllable intervals within sonorous chirps (black bars) (Fig. 4A). (Note that some of the acoustic stimuli presented during the sonorous chirps overlapped with the self-generated sound. Since it was impossible to distinguish these stimuli from the sound produced by the cricket, these stimuli were not evaluated.) In the example presented, the velocity of the RMS response to self-generated sound was 4.1 times greater than the response to a 90 dB SPL sound pulse and was therefore equivalent to a 102.3 dB sound pulse. The RMS response of the tympanic membrane to acoustic stimulation has the same maximum velocity (0.5 mm s^{-1}) during the chirp intervals and during intersyllable intervals (Fig. 4B). However, from these results, it is not possible to determine whether there was a change in responsiveness actually during syllable generation, since responses to stimuli presented in synchrony with the syllables could not be discriminated. In silently singing crickets, we therefore compared the responses during chirp intervals (blue bars) with the responses during the whole chirps (black bars) (Fig. 4C). All the stimuli elicited identical responses of the tympanic membrane (Fig. 4C). The average RMS response reached 0.65 mm s^{-1} both during chirps and during chirp intervals, providing no indication that tympanic membrane responsiveness is modulated during stridulation (Fig. 4D).

Responses of the tympanic nerve at rest and during stridulation

Auditory information is transduced from mechanical oscillations of the tympanic membrane into the action potential discharge rate of primary auditory afferent neurons. The responses of primary auditory afferents were examined by making extracellular hook electrode recordings of the tympanic nerve at rest and during stridulation. Nerve recordings of 15 crickets were combined with recordings of sound and movements of the left wing and left acoustic spiracle. In a resting cricket, primary auditory afferent neurons spiked reliably in response to acoustic stimulation, which leads to patterned nerve activity (Fig. 5A). The averaged, rectified response of the nerve recording was a polyphasic signal with a latency of 5 ms from stimulus onset, lasting for 11.9 ms and peaking at 0.55 mV (Fig. 5B).

In five sonorously stridulating animals, the nerve recording indicated a spiking response of primary auditory afferents that corresponded to the self-generated sound pattern of the cricket (Fig. 6A,B). The nerve recording shows bursts of activity in the rhythm of the syllable pattern, with little or no activity during chirp intervals. The averaged nerve activity corresponded closely to the timing of wing movements and the sound pattern. It consisted of six bursts of activity, which gradually decreased in amplitude, in phase with the six acoustic syllables. The afferent response starts before the first loud syllable is produced. It is possible that the auditory afferents are responding to the very small first opening movement of the wing that causes some sound to be produced (arrows in Fig. 6A,B) or that other mechanosensory afferents that also run in the leg nerve, e.g. from the subgenual organ, are activated with a short latency at the start of stridulation.

To ensure that the primary auditory nerve fibres were not

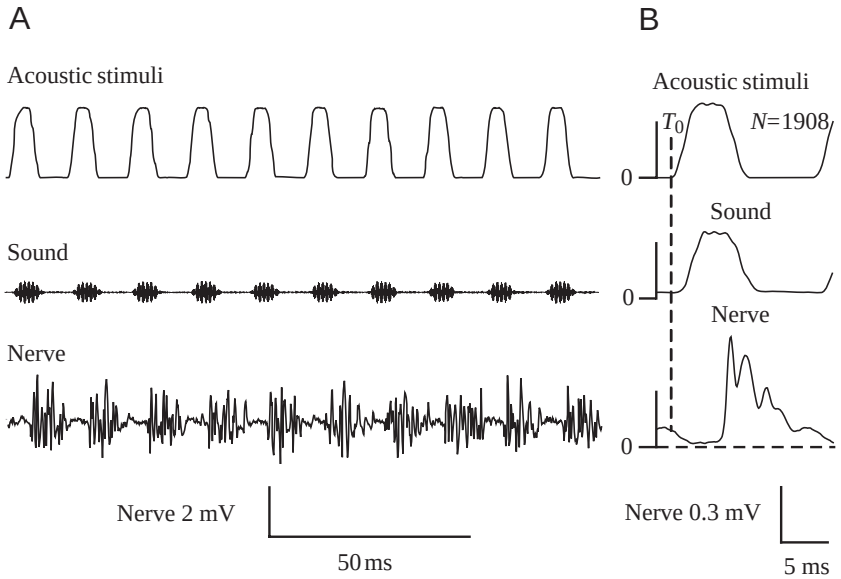

Fig. 5. (A) An extracellular recording of the tympanic nerve afferent response to acoustic stimulation in a resting cricket. (B) The averaged, rectified response of the tympanic nerve to 1908 acoustic stimuli. Nerve, extracellular recording of the tympanic nerve. The stippled, horizontal line denotes the level at which there is no nerve activity. For further details, see Fig. 1.

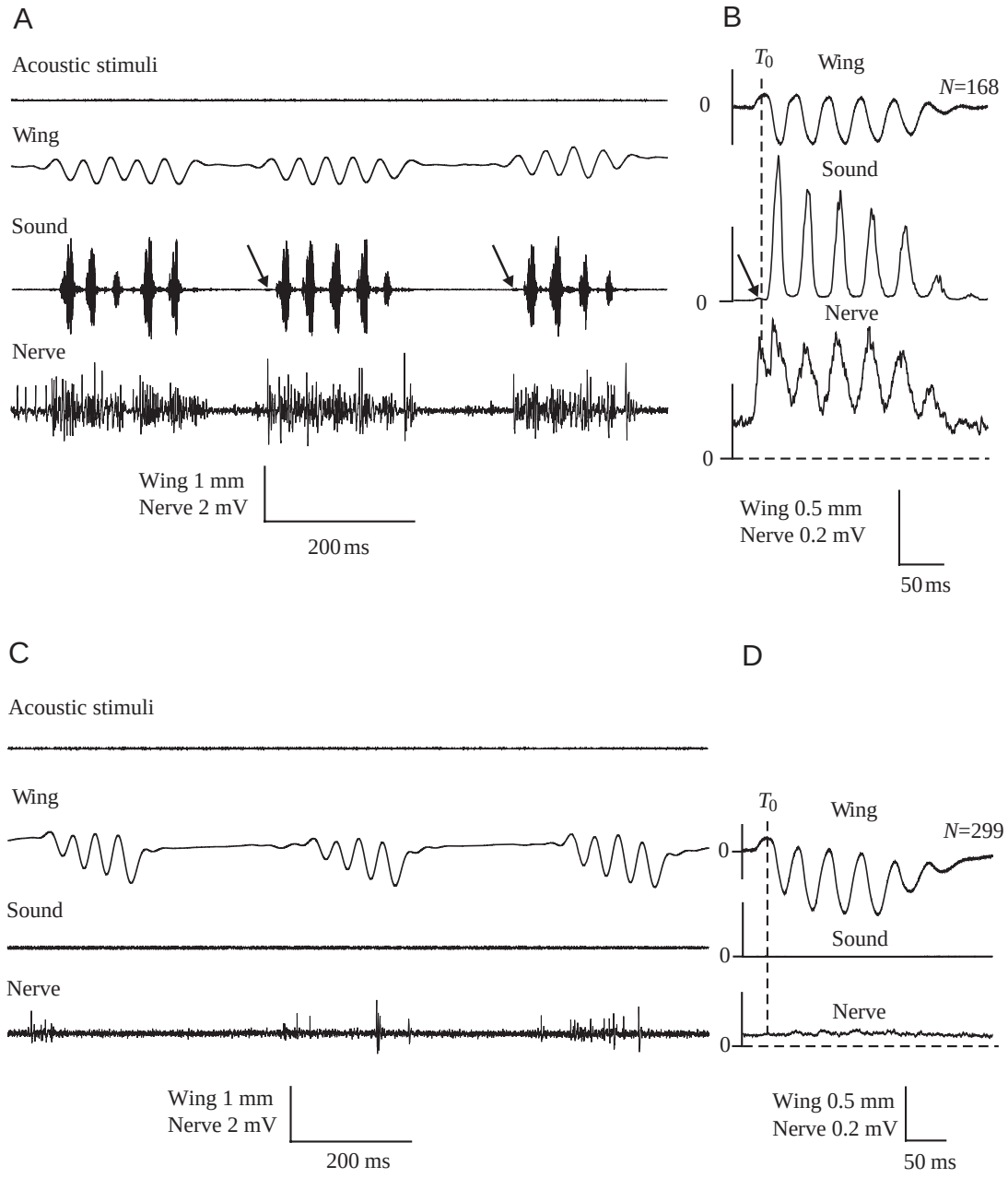

Fig. 6. Tympanic nerve responses during stridulation. (A) Afferents in the tympanic nerve responded during the sonorous chirps but not during the chirp intervals. (B) The averaged wing movement, sound pattern and rectified tympanic nerve response during 168 sonorous chirps. Note that there is afferent activity in the tympanic nerve before the first loud syllable; this is probably a response to the quiet sound, marked by the arrows, produced by the wing opening movement. (C) Response of the tympanic nerve during silent stridulation. (D) The averaged wing movement, sound pattern and rectified tympanic nerve response during 299 silent chirps. Wing, stridulatory wing movements. Nerve, extracellular recording of the tympanic nerve. The stippled, horizontal line denotes the level at which there is no nerve activity. For further details, see Fig. 1.

responding to wing movement and to the underlying motor activity, we removed the right wings of seven crickets, and induced silent stridulation. This also removed any vibratory signals that, during sound production, might spread from the wings through the body of the animal. In silently stridulating crickets, we recorded some minor nerve activity during the chirps (Fig. 6C). The amount of activity we recorded in the tympanic nerve during silent stridulation did not depend on whether it was ipsilateral or contralateral to the remaining wing. The rectified, averaged nerve response confirmed that there was only a very small synchronous response in the tympanic nerve during silent stridulation and, therefore, that the auditory receptors were not fully activated by stridulatory motor pattern generation and wing movements alone (Fig. 6D).

Responsiveness of the tympanic nerve to acoustic stimulation during stridulation

To examine whether the responses of the auditory afferents were modified during the chirps, we presented singing crickets with a continuous sequence of acoustic stimuli. During sonorous stridulation, we compared the responses to stimuli presented during chirp intervals with those in response to stimuli presented during the intersyllable intervals of chirps. When the cricket was singing sonorously, the averaged rectified amplitude of the polyphasic response of the auditory fibres to acoustic stimuli in the chirp intervals was 0.49 mV. In comparison, the peaks of the response during the intersyllable intervals reached only 0.32 mV in amplitude (Fig. 7A,B). At first sight, this may indicate a modulatory effect due to stridulation. However, the afferent responses to the acoustic stimuli in the intersyllable intervals were always preceded by an afferent response to the self-generated sounds of the cricket. Under these circumstances, the auditory afferents will not respond to external sound pulses with the same magnitude because of sensory habituation. To check whether there was modulation of the afferent responses due to chirp pattern generation, we made additional recordings during silent stridulation (Fig. 7C). During silent stridulation, the response of the auditory afferents was the same during the chirps and the chirp intervals. The averaged rectified nerve responses to the acoustic stimuli presented during silent chirps were of exactly the same amplitude (0.52 mV), duration (11.5 ms) and latency (5.4 ms) as the responses in the chirp intervals, indicating that there was no specific modulation of the afferent auditory responsiveness during stridulation (Fig. 7D).

Discussion

Crickets produce loud acoustic signals for communication. One side effect of acoustic communication could be a self-induced desensitisation of the auditory system. To prevent this, the cricket may modulate the responsiveness of its auditory system during sound production. To characterise the responsiveness of the cricket's peripheral auditory system to self-generated sound, we have therefore recorded tympanic membrane oscillations and displacements and tympanic nerve activity during stridulation. We discovered that the responsiveness of the tympanic membrane and tympanic nerve of the cricket is not modulated during stridulation.

Methodological considerations

All crickets were tethered in a natural standing position that allowed all sounds full access to the acoustic spiracles and tympanic membrane. However, as the experiments were not carried out in a sound-proof room and the crickets were surrounded by electrophysiological equipment, sound presented in the far field would have diffracted around the equipment and echoed in the room, which would have led to irregularity in the stimuli. Of paramount importance in these experiments was a constant, repeatable stimulus that would allow an accurate comparison between the acoustic responses during the chirps and the chirp intervals. Stimuli were therefore presented through sound tubes that directed the acoustic stimuli at the tympanic membrane and avoided any echoes. Acoustic stimuli were presented at a high repetition rate to obtain as many data points as possible. The high rate and intensity of the stimuli could have led to habituation in the receptors. However, singing crickets are exposed to even higher sound intensities (102 dB SPL), and tests performed at 75 dB SPL with lower repetition rates yielded the same results (data not shown).

Once tethered, stridulation was elicited by injection of eserine into the frontal protocerebrum. Of the neuroactive substances identified by Otto (Otto, 1978) and Wenzel and Hedwig (Wenzel and Hedwig, 1999) that induce stridulation, we chose eserine because it generated the longest-lasting periods of stridulation (approximately 15 min). It is assumed that eserine induces a build-up of acetylcholine in the brain, exciting identified stridulatory command neurons that descend to the thorax and activate the neural networks responsible for stridulation (Hedwig, 2000b). The amount and quality of the pharmacologically induced songs varied slightly; however, all songs were species-specific to *Gryllus bimaculatus*, and we can assume that the species-specific stridulatory neural networks were activated by pharmacological injection. Systemically applied eserine could have affected auditory afferent responses because it is presumed that insect sensory receptors contain acetylcholine (Sattelle, 1985; Parker and Newland, 1995). However, as the injection (brain) and recording (foreleg) sites were spatially isolated and the auditory responses of the tympanic nerve and tympanic membrane were identical before and after eserine injection, we can conclude that it had no pharmacological effect on the peripheral auditory system.

The role of the acoustic spiracle

The magnitude of the tympanic membrane oscillations in the cricket is a result of auditory input to both its sides. Sound is transmitted to its external side directly from the sound source and to its internal side, *via* the acoustic trachea, from the contralateral tympanic membrane or the two acoustic spiracles. Occlusion of the acoustic spiracles would, therefore, reduce the amount of sound reaching the internal side of the tympanic membrane. Therefore, it is surprising that very little attention has been paid to the movements of the acoustic spiracles during acoustic behaviour in crickets.

Gryllus bimaculatus leaves its acoustic spiracles open for most of the time (Fig. 2) and maintains a constant hearing sensitivity. When the spiracles shut naturally, or were waxed shut, the responsiveness of the tympanic membrane to sound decreased during ventilation. When using crickets with their acoustic spiracles waxed shut, Kleindienst et al. (Kleindienst et al., 1981) noticed a similar modulation in the amount of sound transmitted through the trachea in ventilating crickets. The modulating effect disappeared when the prothorax was opened and could be imitated by pinching the acoustic trachea (Kleindienst et al., 1981). When the acoustic spiracles shut, body movement may deform the acoustic trachea and increase the pressure at the tympanic membrane. This would distort the

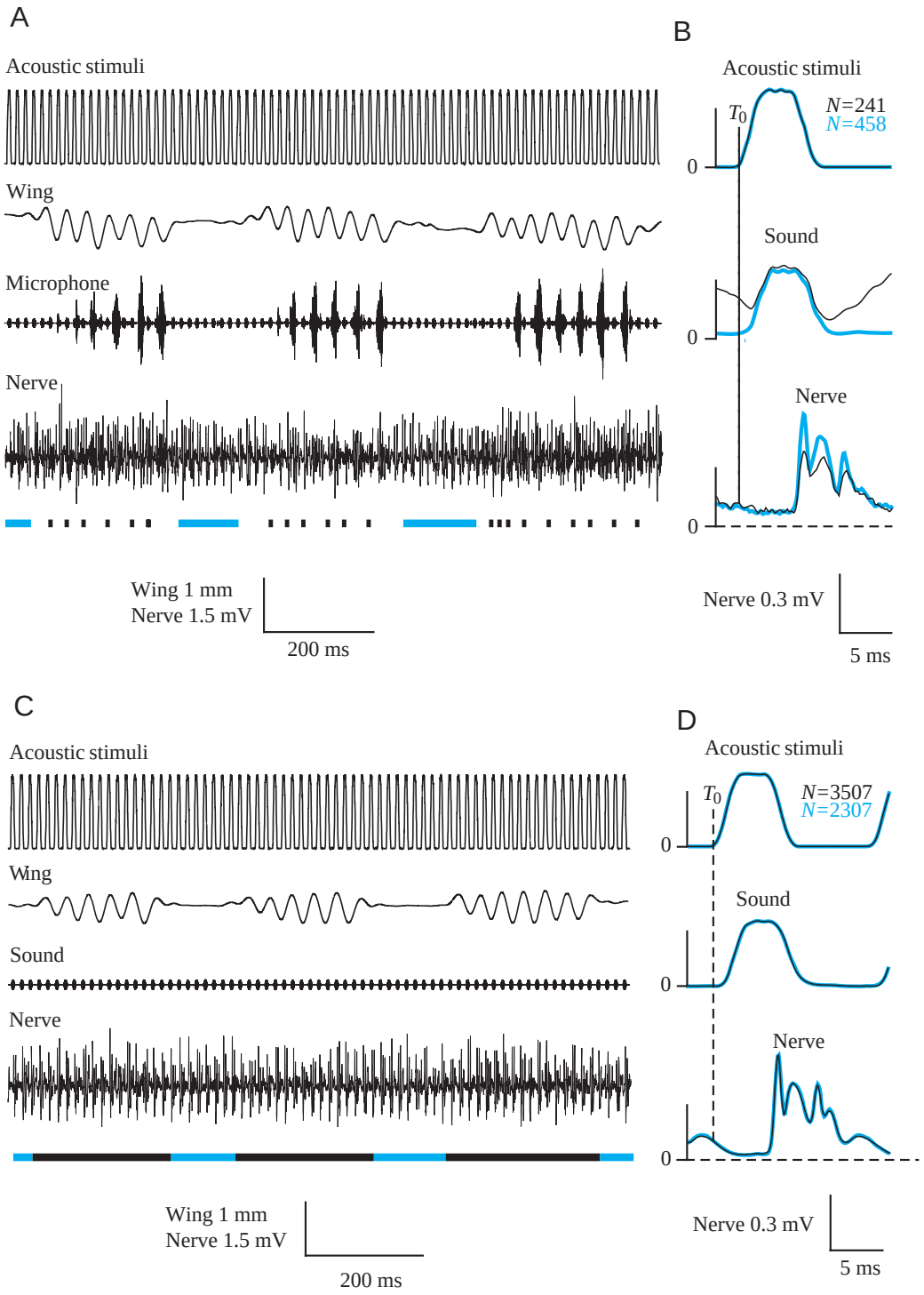

Fig. 7. Tympanic nerve responses to acoustic stimuli during stridulation. (A) Response of afferents in the tympanic nerve to acoustic sound pulses presented during intersyllable intervals (black bars) and chirp intervals (blue bars). (B) The averaged acoustic stimuli, sound pattern and rectified tympanic nerve response during the intersyllable intervals (black line) and during the chirp intervals (blue line). The averaged response of the tympanic nerve during the intersyllable intervals was lower than the response during the chirp intervals (blue line). The averaged sound recording from the intersyllable intervals contains more noise than that from the chirp intervals because sound generated by the cricket was present before and after the intersyllable intervals. (C) The response of afferents in the tympanic nerve to acoustic stimulation during silent chirps (black bars) and chirp intervals (blue bars). (D) The overlaid averages of the sound stimuli, sound pattern and rectified tympanic nerve responses during the silent chirps (black) and the silent chirp intervals (blue) are identical. Wing, stridulatory wing movements. Nerve, extracellular recording of the tympanic nerve. The stippled, horizontal line denotes the level at which there is no nerve activity. For further details, see Fig. 1.

membrane and alter its responsiveness to sound. We propose, therefore, that the acoustic spiracles of the cricket also serve as vents for the release of pressure from the acoustic trachea. This mechanically decouples the responses of the ears of crickets from pressure changes in the ventilatory tracheal system and may serve to prevent the changes in auditory responsiveness due to ventilation, as seen in grasshoppers (Meyer and Hedwig, 1995).

In comparison with the cricket, the peripheral auditory system of the grasshopper has two major anatomical differences. First, the tympanic membranes are located on the first abdominal segment. This rather unstable location means that the membrane is susceptible to deformation during abdominal muscle contraction. Second, at 3–5 kHz, the tympanic membranes are acoustically coupled by a series of closed tracheal sacs (Michelsen, 1971; Michelsen and Rohrseitz, 1995). As these sacs are connected to the tympanic membranes, changes in their internal pressure result in a deformation of the tympanic membrane, which can alter its responsiveness to sound (Meyer and Hedwig, 1995). Thus, unlike that of the cricket, the tympanic membrane of the grasshopper is not decoupled from the tracheal system and responds during types of motor behaviour that could deform the tracheal sacs, including stridulation (Hedwig and Meyer, 1994), ventilation (Hedwig, 1988; Meyer and Elsner, 1995) and even passive leg movements (Lang and Elsner, 1994).

Tympanic membrane oscillation and displacement during stridulation

The sound produced by the singing cricket is reflected in the amplitude and duration of the oscillations of the tympanic membrane (Fig. 3). The velocity of tympanic membrane oscillations was considerably greater than the responses to 90 dB SPL sound pulses (Fig. 4A). This was expected because a recording of cricket song made by Nocke (Nocke, 1972) was over 100 dB SPL; however, the amplitude of the tympanic membrane oscillations could have been augmented as a result of vibrations that occur during sonorous stridulation when the scraper scratches along the file and sets the wings into vibration. These vibrations, which must accompany sound production, might be conducted from the wings *via* the body to the tympanic membrane. No oscillations were recorded from the tympanic membrane during silent chirps in one-winged crickets, suggesting that the ear of the cricket is mechanically isolated from the movement of the wings and thoracic motor machinery, in contrast to the grasshopper, in which the tympanic membrane oscillates during silent stridulation (Hedwig and Meyer, 1994).

To confirm that responses of the tympanic membrane during stridulation were not modulated in comparison with the response in the resting state, we presented 8 ms sound pulses at 4.5 kHz and 90 dB SPL to stridulating crickets. The responses of the tympanic membrane to sound presented during chirp intervals were identical to those in response to sound presented at rest, therefore we compared auditory responses during chirp intervals with responses during chirps. The tympanic membrane oscillations, produced in response to acoustic stimulation, had the same amplitude during the chirps and the chirp intervals (Fig. 4). In comparison, the frog and cicada both display low-frequency displacements of the tympanic membrane during sound production, and both show a modulation in responsiveness (Narins, 1992; Hennig et al., 1994). The reduction in responsiveness of the tympanic membrane of the frog is due to the low-frequency displacements of the tympanic membranes caused by changes in the internal pressure of the closed, air-filled tubes that connect the inner surfaces of the tympanic membranes (Narins, 1992). The cicada, in contrast, has direct control over displacements of the tympanic membrane. It folds its tympanic membranes during sound production and high-intensity sound stimulation, thus increasing the threshold of auditory responses in the afferents by 20 dB SPL (Hennig et al., 1994). The tympanic membrane of the grasshopper shows a complex pattern of displacements imposed on the tympanic membrane during stridulation, but this does not modulate the sensitivity of the tympanic membrane (Hedwig and Meyer, 1994).

Unlike those of the grasshopper, cicada and frog, the tympanic membrane of the cricket is not displaced during sound production. We propose that this is the result of two anatomical characteristics of the peripheral auditory system of the cricket. First, the tympanic membrane of the cricket is located on the relatively rigid tibiae of the foreleg and is, therefore, not as susceptible to displacement during body movement. Second, the open acoustic spiracles may act as air vents that maintain a constant pressure in the acoustic trachea during stridulation and, thereby, prevent tympanic membrane displacement.

Tympanic nerve activity during stridulation

The summed response of the tympanic nerve during stridulation closely corresponds to the timing of the sound pattern produced by the cricket (Fig. 6B). Although we have attributed this response to the primary auditory afferent activity, some of it may have been the result of mechanosensory and vibration receptor activity, which is also present in the tympanic nerve. These receptors project from the subgenual organ along the tympanic nerve and may respond both to vibrations conducted *via* the exoskeleton during sound production and to air-borne sound (Kühne et al., 1984). During silent stridulation, there was some minor tympanic nerve activity during the chirps (Fig. 6C). This activity may also be due to the activation of mechanosensory afferents by wing movements or of auditory receptors by background noise.

Acoustic stimuli were presented during the chirps and chirp intervals to assess further the responsiveness of the tympanic nerve of the cricket. Compared with the response during the chirp interval, the rectified response of the tympanic nerve was slightly smaller during the intersyllable intervals during sonorous stridulation but, crucially, not during the silent chirps (Fig. 8B,D). Any change in response of the auditory afferents during sonorous chirps was not, therefore, due to a mechanism associated with chirp pattern generation but was probably the

result of receptor habituation to loud self-produced sounds (Esch et al., 1980; Ocker and Hedwig, 1993; Givois and Pollack, 2000).

In contrast to the cricket, the tympanic nerve of the grasshopper and cicada responds during both silent and sonorous stridulation (Hedwig and Meyer, 1994; Hennig et al., 1994). The rectified response of the auditory afferents of the grasshopper to acoustic stimuli during stridulation is lower in amplitude than in resting grasshoppers because the background activity of the receptors masks the response to the stimuli (Hedwig and Meyer, 1994). In the ascending auditory pathway, a reduction in synchronous receptor activity causes a reduction in the excitation of auditory interneurons during certain phases of stridulation (Hedwig, 1986; Wolf and von Helversen, 1986; Hedwig, 1990; Hedwig and Meyer, 1994). The cicada also shows a reduction in the response of its auditory afferents to sound stimuli during stridulation; however, it actively controls the responsiveness of its tympanic membrane during stridulation (Hennig et al., 1994). We conclude that, unlike that of the grasshopper and cicada, the responsiveness of the auditory receptors of the cricket is not impaired or modulated during stridulation.

Concluding remarks and future work

Why does the cricket *Gryllus bimaculatus* maintain peripheral auditory responsiveness despite producing such loud sounds? One answer could be that reafferent auditory information helps to stabilise and fine-tune the ongoing stridulatory motor pattern. In fact, Bennet-Clark (Bennet-Clark, 1987) suggested that the mole cricket *Scapteriscus acletus* optimises the acoustic properties of its burrow by repeatedly monitoring the power output of its song while building the burrow. In *Gryllus bimaculatus*, however, recent biophysical evidence suggests that this species does not require reafferent auditory feedback to determine its major song parameters (Prestwich et al., 2000). Even if peripheral auditory sensitivity were reduced during singing, reafferent information from the cerci and wing hair fields would still be present and could help regulate singing (Dambach et al., 1983; Elliott and Koch, 1983).

The maintenance of auditory responsiveness during sound production would allow male crickets to listen to conspecific sounds and perhaps to noisy predators. Male *Gryllus bimaculatus* form calling aggregations within which they are separated by 0.5–40 m (average 2 m) (Simmons, 1988). The ability to hear calling conspecifics during singing allows male *Gryllus bimaculatus* to maintain a fixed distance from each other and to defend their territory from encroaching conspecific singers (Simmons, 1988). Like many Orthoptera, singing crickets (*Gryllus campestris*, *Acheta domesticus*) will modulate their singing patterns when presented with conspecific sounds during the chirp intervals (Heiligenberg, 1969; Jones and Dambach, 1973). This behaviour relies on the ability of the cricket to hear during stridulation and could either lead to alternating singing (as in acridids; Minckley et al., 1995) or synchronised singing (as in tettigoniids; Greenfield, 1994), although to our knowledge this has not been reported for *Gryllus bimaculatus*.

Given that the presentation of loud sounds results in a prolonged inhibition of cricket auditory interneurons (Pollack, 1988), it is curious that crickets maintain peripheral auditory responsiveness during stridulation. Behavioural studies have demonstrated that stridulating crickets maintain auditory responsiveness during chirp intervals (Heiligenberg, 1969; Jones and Dambach, 1973), and, therefore, the central auditory system cannot be completely desensitised by sound production. One method, more precise than peripheral filtering, of preventing auditory desensitisation may be to modulate the responses of central auditory neurons during stridulation. We are currently testing this hypothesis.

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