

PHASIC ACTION OF THE TENSOR MUSCLE MODULATES THE CALLING SONG IN CICADAS

P. J. FONSECA¹ AND R. M. HENNIG^{2,*}

¹*Departamento de Zoologia e Antropologia da Faculdade de Ciências de Lisboa, Bloco C2, Campo Grande, 1700 Lisboa, Portugal* and ²*Max-Planck-Institut für Verhaltensphysiologie, D-82319, Seewiesen, Germany*

Accepted 25 March 1996

Summary

The effect of tensor muscle contraction on sound production by the tymbal was investigated in three species of cicadas (*Tettigetta josei*, *Tettigetta argentata* and *Tympanistalna gastrica*). All species showed a strict time correlation between the activity of the tymbal motoneurone and the discharge of motor units in the tensor nerve during the calling song. Lesion of the tensor nerve abolished the amplitude modulation of the calling song, but this modulation was restored by electrical stimulation of the tensor nerve or by mechanically pushing the tensor sclerite.

Electrical stimulation of the tensor nerve at frequencies higher than 30–40 Hz changed the sound amplitude. In *Tett. josei* and *Tett. argentata* there was a gradual increase in sound amplitude with increasing frequency of tensor nerve stimulation, while in *Tymp. gastrica* there was a sudden reduction in sound amplitude at stimulation frequencies higher than 30 Hz. This contrasting effect in *Tymp. gastrica* was due to a bistable tymbal frame. Changes in sound pulse

amplitude were positively correlated with changes in the time lag measured from tymbal motoneurone stimulation to the sound pulse. The tensor muscle acted phasically because electrical stimulation of the tensor nerve during a time window (0–10 ms) before electrical stimulation of the tymbal motoneurone was most effective in eliciting amplitude modulations.

In all species, the tensor muscle action visibly changed the shape of the tymbal. Despite the opposite effects of the tensor muscle on sound pulse amplitude observed between *Tettigetta* and *Tympanistalna* species, the tensor muscle of both acts by modulating the shape of the tymbal, which changes the force required for the tymbal muscle to buckle the tymbal.

Key words: tymbal, tymbal muscle, sound modulation, cicada, *Tettigetta* sp., *Tympanistalna gastrica*.

Introduction

Cicadas produce the sound patterns of their songs by buckling a pair of tymbals in the first abdominal segment (Pringle, 1954a; Moore and Sawyer, 1966; Reid, 1971; Young and Bennet-Clark, 1995). The tymbal is a cuticular membrane whose stiffness depends mainly on the number of ribs it contains (Popov, 1975) and the amount of embedded resilin (Young and Bennet-Clark, 1995). Contraction of the large tymbal muscles causes the tymbal ribs to buckle inwards and the tymbal then jumps back because of its elasticity. Inward and outward movements of the tymbal may produce sound. The sound pulses generated by one tymbal muscle contraction are defined as a tymbal cycle. Amplitude modulation of tymbal cycles can be attributed to the action of two systems: the tensor muscle exerts a force on the tymbal frame and thus modulates the stress of the tymbal and its resulting sound output (Pringle, 1954a; Simmons and Young, 1978); in addition, the largely

air-filled abdomen may influence sound amplitude as a result of changes in the radiation or resonance properties of the cicada body (Pringle, 1954a; Bennet-Clark and Young, 1992; Fonseca and Popov, 1994).

Current understanding of tensor muscle action comes mostly from studies in which the tensor muscle was electrically stimulated and the effect of the muscle contraction on the amplitude of the sound pulses produced was monitored. Such experiments suggest that a tonic contraction of the tensor muscle increases the stiffness of the tymbal (Pringle, 1954a; Hagiwara, 1956; Simmons and Young, 1978). The higher force then required to buckle the tymbal results in higher-amplitude sound pulses with a concurrent change in the timing of the tymbal sound with respect to the tymbal muscle contraction (Pringle, 1954b; Simmons and Young, 1978). Overstressing of the tymbal due to maximum contraction of the tensor muscle

*Present address: Abteilung Verhaltensphysiologie, Institut für Biologie, Humboldt Universität zu Berlin, Invalidenstraße 43, D-10115 Berlin, Germany.

may also cause a reduction in sound amplitude (Hennig *et al.* 1994). Most of these studies have recorded tensor muscle activity during the alarm or distress call of the males and only Weber *et al.* (1988) have succeeded in recording tensor muscle activity during calling song production.

Cicadas possess different tymbal types which show rather distinct tymbal cycles due to their morphology and stiffness (Popov, 1975). To date, most studies on tensor muscle function have been concerned with cicadas which produce several sound pulses in a tymbal cycle due to the sequential buckling of the tymbal ribs. However, there is a group of cicadas (*Tettigetta* and *Tympanistalna*, Fonseca, 1991; *Cicadetta*, Popov, 1975) in which all the tymbal ribs buckle simultaneously. These cicadas show very fast, large amplitude modulations in their calling songs (within less than 10 ms) which cannot be accounted for either by a tonic contraction of the tensor muscle or by a positional change of the abdomen.

There is, however, indirect evidence that the tensor muscle is indeed responsible for the fast and large amplitude modulations observed in these species (Fonseca, 1991). We therefore compared the function of the tensor muscle in several species by recording tensor muscle activity during normal calling song production and by investigating the effects on the sound modulation elicited through electrical stimulation of the tensor nerve. It is shown that the tensor muscle is responsible for the fast amplitude modulations observed in the calling song and that the action of the tensor muscle is phasic.

Materials and methods

Animals

The cicada species *Tettigetta josei* (Boulard), *Tettigetta argentata* (Olivier) and *Tympanistalna gastrica* (Ståhl) were caught on the southwest coast of Portugal in June 1992. The animals were kept on a netted feeding plant in a cool room at 10–15 °C.

Morphology of the sound-producing structures

Adult cicadas were killed and fixed in a solution modified after Carnoy for 24 h at room temperature (Bock, 1987) and then preserved in 70% ethanol. Drawings were made from observations using a Wild M5A stereomicroscope equipped with a drawing tube, using whole animals as well as longitudinal and transverse sections of the cicada bodies.

Preparation of the cicadas for nerve recording and electrical stimulation

A male cicada, with its wings, legs and mouthparts removed, was waxed to the end of a slender rod (110 mm long, 2 mm diameter) attached to a magnetic stand. Two electrodes (minutien insect pins) were implanted into the head near the medial edge of the compound eye. The mesosternum was removed to expose the nerves exiting from the metathoracic abdominal ganglionic complex (MAC). The abdominal nerves were cut in order to preclude movements of the abdomen which might influence sound radiation. Both the tensor nerve

and auditory nerve on one side were lifted on double silver hook electrodes (diameter 50 µm) for recording and electrical stimulation (Fig. 1). In all three species, the auditory nerve carries both the auditory receptor fibres and the tymbal motoneurone. The dissected area was kept moist with insect Ringer until both nerves had been insulated with a mixture of Vaseline and mineral oil. The cicada was earthed by a wire inserted into the mesothoracic leg stump. All experiments were conducted at room temperature (24±2 °C).

Sound monitoring

Hard surfaces close to the animal were covered with sound-absorbing material (cotton wool and Illbruck Super Waffel pieces) in order to reduce echoes. The sound generated by the cicada was monitored using a microphone (Sennheiser MKE-2) placed approximately 10 cm away from the animal and amplified using a UHER 4200 tape recorder (the sound pressure measured with this system was constant within ±3 dB in the range 0.5–18 kHz). Sound, nerve activity and electrical stimulation signals were stored on magnetic tape with a DAT recorder (TEAC 100T).

Electrical stimulation

For brain stimulation, pulsed electrical stimuli (pulse duration 1 ms; amplitude 1–10 V; stimulus frequency 10–60 Hz from a battery-powered stimulus isolation unit) were applied through the electrodes inserted in the head. Stimulation for 1–2 s was in most cases sufficient to elicit singing with a

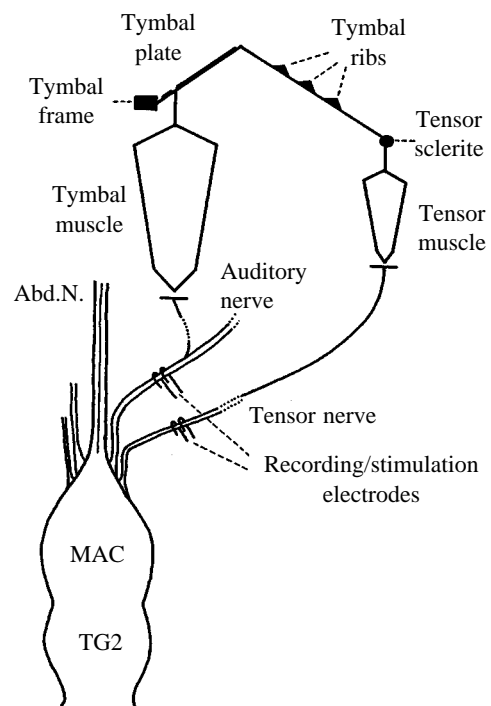


Fig. 1. Schematic drawing of the sound-producing tymbal and the neuromuscular apparatus investigated by electrical nerve stimulation. The auditory nerve contains the tymbal motoneurone. Abd.N., abdominal nerves; MAC, metathoracic abdominal ganglionic complex; TG2, second thoracic ganglion.

pattern similar to the calling song and starting within a few seconds to 2 min after the stimulation ended. Electrical stimulation of the auditory and tensor nerves was *via* insulated battery units and the double silver hook electrodes (Fig. 1). The duration of the stimulation pulses was set at 0.5 ms and the frequencies used ranged from 5 to 50 Hz (occasionally 100 Hz) for the auditory nerve (tymbal motoneurone) and from 10 to 200 Hz for the tensor nerve.

Visual observation of the changes induced in the tymbal and tymbal frame during electrical stimulation of the tensor nerve

Tensor muscle contraction was induced by tensor nerve stimulation, usually at approximately 100 Hz. The resulting morphological changes were observed using a stereomicroscope (WILD M5A) and noted on a drawing of the tymbal. The direction of movement (inward or outward) as well as the magnitude of the displacement (classified as small, medium or large) was noted.

Mechanical pushing of the tensor sclerite during sound production

The effect of tensor muscle contraction was simulated by pushing the tensor sclerite using the smoothed tip of an insect pin (see Fig. 1). The pin was mounted on a holder and advanced using a micromanipulator. The effects on the sound pulses were recorded both during the calling song and during electrical stimulation of the tymbal motoneurone (i.e. auditory nerve).

Measurements of the force necessary to buckle the tymbal inwards both with and without tensor nerve stimulation

The tymbal was loaded with a force by advancing a spring mounted on a micromanipulator, both with and without an electrically induced tensor muscle contraction. The tip of the spring, 100 µm in diameter, was placed on the apodeme pit where the tymbal muscle apodeme attaches (see Fig. 2; Young and Bennet-Clark, 1995). The force was increased steadily by advancing the micromanipulator until buckling of the tymbal occurred, usually accompanied by sound production. The force was then determined from the micromanipulator reading, which was compared with a calibration curve constructed using a balance. A force of 1 mN deformed the spring by about 0.75 µm, which is at least 10 times greater than the observed deformation of the tymbal plate. The size of the tip of the spring, the position over the tymbal where the force was applied and the angle of incidence of this force had to be chosen carefully (see also Young and Bennet-Clark, 1995). Applying the force in other positions on the tymbal plate or modifying the angle usually resulted in no measurable differences between the force applied before and after tensor muscle contraction.

Data analysis

Data analysis was carried out using a Macintosh computer equipped with appropriate software (SuperScope). Chart prints were made on an eight-channel chart recorder (Picker Uniscript

UD210), after transferring the data from the DAT recorder to a Racal Store 4DS.

Nomenclature

Sound pulses are referred to as IN sound pulses when they were produced during inward buckling of the tymbal and as OUT sound pulses when they were produced during outward movement of the tymbal. The term 'time lag' refers to the time elapsed between electrical stimulation of the tymbal motoneurone (in the auditory nerve) and the occurrence of the resulting IN or OUT sound pulse.

Results

Morphology of the sound-producing apparatus

The sound-producing apparatus of the males of *Tett. josei* and *Tymp. gastrica* (Fig. 2) is composed of the tymbals, which are driven by the powerful tymbal muscles, and the tensor muscle, which inserts at the anterior tymbal frame (see also Fig. 1 for a schematic view). A large tracheal air sac lines the tymbals, the tympana and the folded membranes and exits through the metathoracic spiracles (Fig. 2B). The wall of the abdomen is internally lined by a relatively thick layer of tissue in *Tett. josei*, but only a thin layer in *Tymp. gastrica*.

The tymbal of *Tett. josei* shows a convex region anteriorly with three long and two short sclerotized ribs and a flatter tymbal plate posteriorly (Fig. 2A). The two most posterior long ribs join dorsally whereas the third, more anterior rib is separated from the two long ones. The tymbal buckles along the short ribs. The tymbal muscles arise ventrally from the chitinous V and insert through a tendinous apodeme on the dorsal portion of the tymbal plate. From outside, the tymbal muscle apodeme at the tymbal plate is easily recognized as a small invagination – the apodeme pit. The tymbal frame has a relatively large tensor sclerite anteriorly which is surrounded by a flexible membrane, allowing movement (Fig. 2A). The stout and strong tensor muscle arises from a sclerotized metathoracic ridge just ventrally to the tymbal and anteriorly to the folded membrane and inserts at the tensor sclerite (Fig. 2B). Visual observations revealed that tensor muscle contraction moves the whole tensor sclerite inwards, especially at the anterior edge (arrows in Fig. 2A).

In *Tymp. gastrica*, the convex tymbal area possesses four long sclerotized ribs, but no short ones (Fig. 2C). The posterior two ribs join dorsally. The posterior three ribs are slender in the medial region of the tymbal, where the tymbal buckles. The most anterior fourth rib is attached to the tymbal frame. The tensor sclerite is dorsally and anteriorly, but not ventrally, surrounded by a flexible membrane. Visual observations showed that tensor muscle contraction moved the tensor sclerite inwards, mostly at the anterior and dorsal edge (arrow in Fig. 2C).

*Function of the tensor muscle in *Tettigetta josei**

The tymbal of *Tett. josei* produces one IN and one OUT sound pulse in a tymbal cycle (Fig. 3A). The calling song

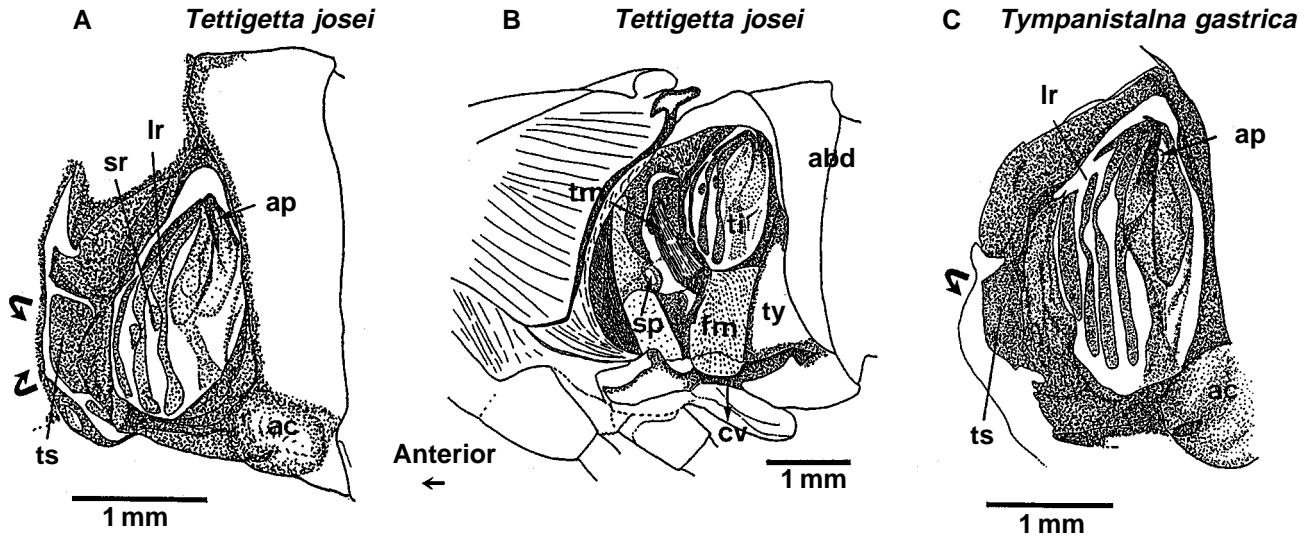


Fig. 2. Sound-producing apparatus and tymbals of male *Tettigetta josei* (A,B) and the tymbal of *Tympanistalna gastrica* (C). (A,C) External view of the tymbals. (B) Drawing of the tymbal apparatus and selected structures as revealed by a longitudinal vertical section of a *Tett. josei* male with the tymbal muscle removed. The sound-producing apparatus of *Tymp. gastrica* is rather similar in size and structure to that of *Tett. josei* and is therefore omitted here. Anterior is to the left in all drawings. Curved arrows in A and C indicate the direction of movement of the anterior part of the tensor sclerite. abd, anterior part of the abdominal cavity; ac, auditory capsule; ap, apodeme pit; cv, chitinous V; fm, folded membrane; lr, long ribs; sr, short ribs; ti, tymbal; tm, tensor muscle; ts, tensor sclerite; ty, tympanum.

consists of echemes, each of which is defined by two consecutive tymbal muscle contractions (Fig. 3; for details, see Fonseca, 1991). Pulsed electrical stimulation of the brain elicited this pattern reliably while recordings from the auditory nerve containing the tymbal motoneuron and the tensor nerve were made (Fig. 3A). In an echeme of *Tett. josei*, the first IN sound pulse was very soft while the second IN pulse was very loud. The tymbal motoneuron (large potential in the middle trace of Fig. 3A) showed two action potentials per echeme which were accompanied by the activity of several units in the tensor nerve recording spaced between the tymbal motoneuron spikes (Fig. 3A). Cutting the tensor nerve abolished the loud IN sound pulses (Fig. 3B), while electrical stimulation of the tensor nerve resulted in IN sound pulses of large amplitude (Fig. 3C). The amplitude of the OUT sound pulses is not modulated during the calling song (Fig. 3A) and electrical stimulation of the tensor nerve did not influence the OUT pulse amplitude (Fig. 3C).

The tensor nerve not only innervates the tensor muscle, but

also the intersegmental dorsal muscles which control lift of the abdomen (Pringle, 1954a; Simmons and Young, 1978). Furthermore, the tensor nerve contains a large number of mechanoreceptor axons from the tensor chordotonal organ which may provide sensory feedback about tymbal buckling (Young, 1975). The following controls ensured that the observed effects were solely due to the contraction of the tensor muscle. (1) Cutting the tensor nerve between the MAC and the stimulation electrodes did not affect the changes in sound-pulse amplitude due to electrical nerve stimulation, and the timing of the central nervous motor output was unchanged

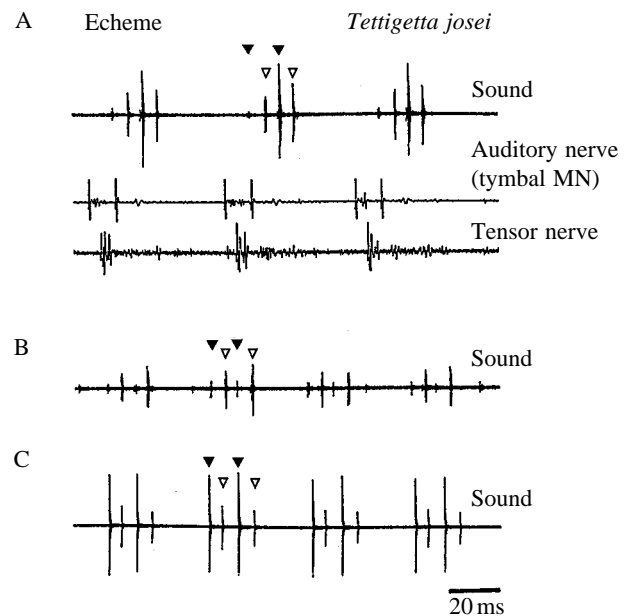


Fig. 3. Sound recordings and recordings from the auditory nerve and the tensor nerve of *Tettigetta josei* during calling song production elicited by electrical brain stimulation. The auditory nerve contains the tymbal motoneuron (MN). (A) During the calling song (upper trace, sound pulses; IN, filled symbols; OUT, open symbols), the tymbal motoneuron showed two large action potentials per echeme (middle trace) which were accompanied by the activity of several units in the tensor nerve recording (bottom trace). (B) Lesion of the tensor nerve resulted in a reduction in amplitude of the second IN pulse, while OUT pulses were less affected. (C) Electrical stimulation of the tensor nerve at frequencies higher than 100 Hz resulted in uniformly loud IN pulses.

when the tensor nerve was cut distally to the recording electrodes (see Fig. 1). (2) Mechanical pressure on the sclerite alone had the same effect on sound-pulse amplitude as electrical stimulation of the tensor nerve. Thus, contraction of the tensor muscle alone was responsible for the observed amplitude modulations.

Electrical stimulation of the tymbal motoneurone and the tensor nerve (see Fig. 1) showed that the effect of tensor muscle contraction on the sound-pulse amplitude was gradual (Fig. 4) and dependent on the frequency of electrical stimulation. Beginning at a tensor nerve stimulation frequency of approximately 40 Hz, there was an increase in amplitude mainly of the IN sound pulses, up to frequencies higher than 100 Hz (Fig. 4A). The changes in sound amplitude were accompanied by changes in the time lag between electrical stimulation and the sound pulse (Fig. 4B). The effect of tensor muscle contraction was independent of the rate of tymbal motoneurone stimulation (at 5, 20 or 50 Hz). The amplitude change induced by tensor muscle contraction was greater than

20 dB, and the amplitude and time lag of the IN pulses showed a positive correlation (Fig. 4C). Electrical stimulation of the tensor nerve had only a small effect on the OUT pulse amplitude (Fig. 4C). Examination of the relative timing of tensor nerve and tymbal motoneurone stimulation revealed a time window (-10 to 0 ms) during which a single stimulation of the tensor nerve strongly affected the IN pulse amplitude (Fig. 5). The amplitude effect of the time window accounted for approximately 30% of the maximal amplitude modulation observed in an individual animal. The time lag between auditory nerve and tensor nerve stimulation for the results in Fig. 4A,B had been chosen such that, at low stimulation frequencies of the tensor nerve, the phasic effect (Fig. 5) was excluded.

The observed increase in the amplitude and the time lag of the IN pulse due to electrical stimulation of the tensor nerve can be interpreted in the following way. Contraction of the tensor muscle increases the convexity of the tymbal and thus the tymbal muscle takes more time to overcome the increased

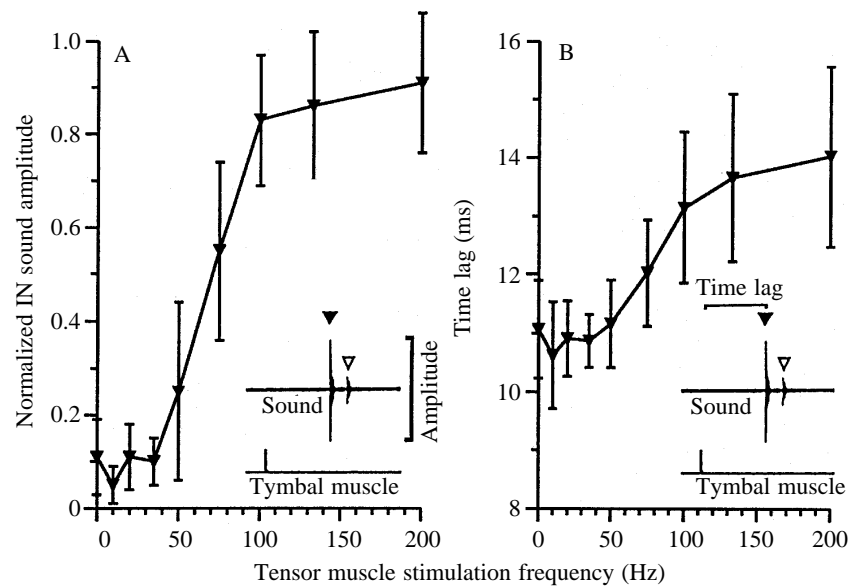
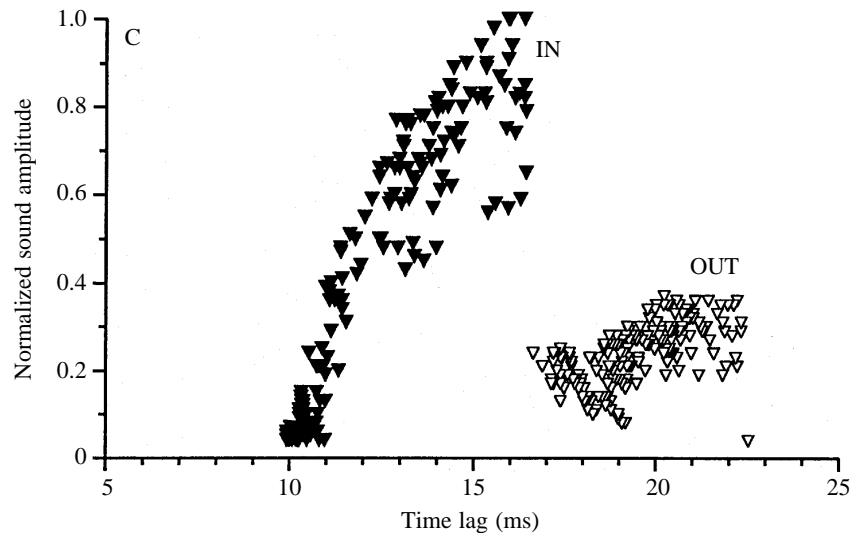


Fig. 4. Electrical stimulation of the tymbal motoneurone (20 Hz) and concomitant electrical stimulation of the tensor nerve of *Tettigetta josei* at frequencies up to 200 Hz affects sound pulse amplitude and time lag. Insets show sound recordings of a tymbal cycle with electrical stimulation of the tensor nerve and illustrate how sound amplitude and time lag were determined for IN sound pulses (filled symbols). Open symbols represent OUT sound pulses. (A) Amplitude and (B) time lag of IN sound pulses increased with increasing stimulation frequencies of the tensor nerve. Mean values \pm S.D. from five males. (C) Relationship between sound amplitude and time lag of IN and OUT sound pulses obtained from one tensor nerve stimulation series. Amplitudes are normalized to the amplitude of the loudest IN pulse. IN (filled symbols), OUT (open symbols); data from one male.



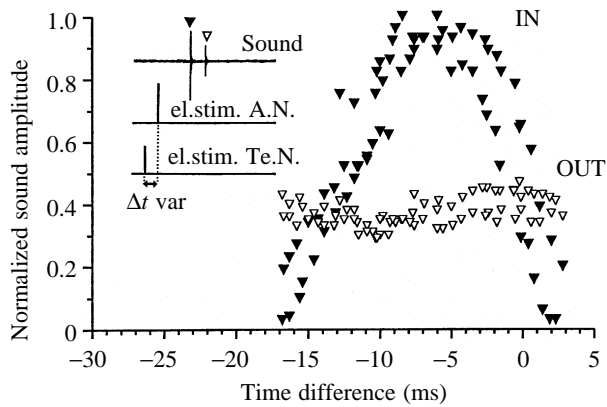


Fig. 5. Dependence of amplitude of IN and OUT sound pulses on the relative timing of tymbal motoneurone (A.N.) and tensor nerve (te.N.) stimulation (el.stim.) in *Tettigetta josei*. The inset defines the time difference (Δt var) between the two stimulus pulses. Stimulation of the tensor nerve from 10 ms before the tymbal motoneurone stimulation (shown as negative values) to simultaneous stimulation (i.e. 0 ms) was effective in changing the amplitude. Data points correspond to single sound pulses from tensor nerve stimulation at less than 40 Hz. Amplitudes are normalized to the amplitude of the loudest IN pulse under these stimulation conditions. Note that the highest amplitudes in this diagram correspond to about one-third of the maximal amplitude modulation. IN, filled symbols; OUT, open symbols; data from two males.

mechanical resistance of the tymbal, which will produce louder sound pulses when buckled. Hence, an increase in the force required to buckle the tymbal is expected. Measurements of this force were conducted by placing the tip of a spring on the apodeme pit of the tymbal plate and by advancing the spring until the tymbal buckled. Without electrical stimulation of the tensor nerve, a force of 1.0 mN was necessary to buckle the tymbal of *Tett. josei* (Fig. 6). However, electrical stimulation of the tensor nerve increased the force which had to be applied to the apodeme pit in order to buckle the tymbal. This force changed in the same frequency range of tensor nerve stimulation as previously recorded for the amplitude and time lag of the IN pulses (see Fig. 4).

Function of the tensor muscle in *Tettigetta argentata*

The tymbal cycle in *Tett. argentata* produces one IN and one OUT sound pulse (Fig. 7). The calling song differs from that of *Tett. josei* in that each echeme is defined by three consecutive contractions of the tymbal muscles (Fig. 7; Fonseca, 1991). In contrast to the echemes of *Tett. josei*, values of intermediate amplitude were observed (e.g. for the second IN sound pulse in each echeme, Fig. 7) and the amplitude of the OUT pulses was also modulated (compare the first and second OUT pulse in each echeme, Fig. 7). Recordings from the tensor nerve revealed units which were active just prior to the first tymbal motoneurone spike, but also at the second tymbal motoneurone spike (Fig. 7) in an echeme. A set of experiments identical to those performed on *Tett. josei* (see Figs 4, 5) gave similar results, i.e. IN pulse amplitude was

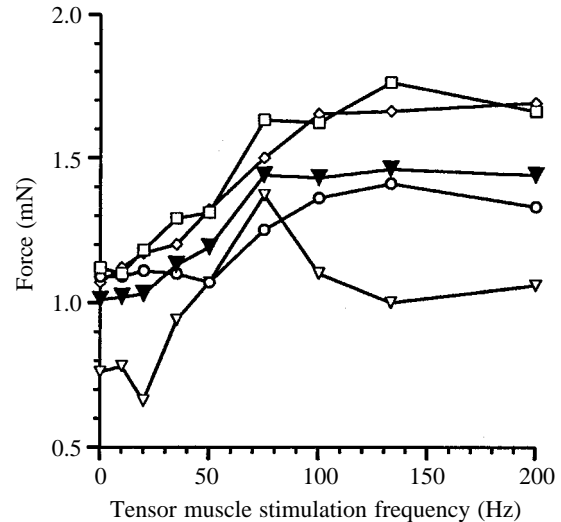


Fig. 6. Force required to buckle the tymbal of *Tettigetta josei* under different magnitudes of tensor muscle contraction induced by electrical stimulation. Higher stimulation rates of the tensor nerve resulted in greater forces. Mean values (filled symbols) calculated from four tymbals of two males (open symbols) are shown.

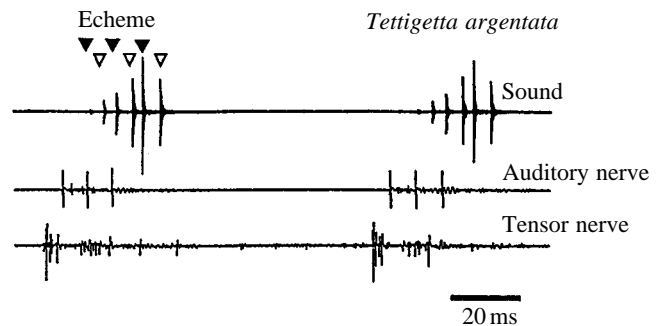
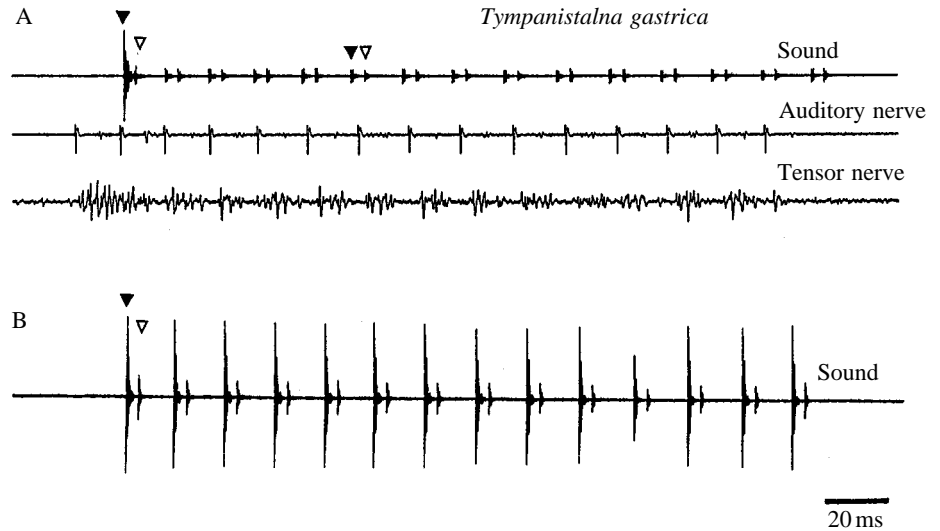


Fig. 7. Sound recordings and recordings from the auditory nerve containing the tymbal motoneurone and from the tensor nerve of *Tettigetta argentata* during calling song production (upper trace, sound pulses; IN, filled symbols; OUT, open symbols). The tymbal motoneurone showed three large action potentials per echeme (middle trace). Several units in the tensor nerve showed a constant activity pattern per echeme (bottom trace). Recording from a male with only one tymbal intact.

dependent on the frequency of electrical stimulation of the tensor nerve, a positive relationship existed between time lag and IN pulse amplitude, and a time window within which a single tensor stimulation pulse elicited a large effect on the sound-pulse amplitude was observed. While the principal mechanism of tensor muscle action appeared to be the same for both species, some distinct differences were also observed, probably due to the difference in stiffness of the tymbal: (1) the OUT pulse amplitude also increased with the frequency of tensor nerve stimulation, (2) the effective time window was larger (-25 to $+5$ ms), although the maximal effect was also observed from -10 to 0 ms, and (3) the force necessary to buckle the tymbal was higher (from 2.2 mN without

Fig. 8. Sound recordings and auditory and tensor nerve recordings during calling song production from *Tympanistalna gastrica* with only one tymbal (upper trace, sound pulses; IN, filled symbols; OUT, open symbols). During an echeme (one echeme is shown in the top trace), the tymbal motoneurone (large potential in the auditory nerve recording, middle trace) showed a series of action potentials which were accompanied by the activity of several units in the tensor nerve recording (bottom trace). (B) Lesion of the tensor nerve resulted in an increase in the IN sound amplitude throughout the calling song, although OUT sound amplitude was unaffected.



stimulation of the tensor nerve to 2.6 mN at a stimulation frequency of 100 Hz).

Function of the tensor muscle in *Tympanistalna gastrica*

The tymbal produces one IN and one OUT sound pulse by buckling during one contraction of the tymbal muscle (Fig. 8A). In a type 1 echeme of the calling song, the amplitude of the first IN pulse of both tymbals is high, while all following OUT and IN pulses are of the same reduced loudness (Fig. 8A; see Fonseca, 1991, for details of the calling song). *Tymp. gastrica* shows three types of echeme, with type 1 being the most common and typical. Nerve recordings showed that bursts of several units in the tensor nerve occurred between the action potentials of the tymbal motoneurone (Fig. 8A). The IN pulses produced by the tymbal when the tensor muscle was disabled were always as loud as the first IN pulse in an echeme (Fig. 8B). The amplitude of the OUT pulses was not affected by the lesion of the tensor nerve. The same control experiments for influences other than the tensor muscle were conducted as in *Tett. josei*, but again there was no evidence for the action of any additional system.

In order to study the effect of tensor muscle contraction on the sound-pulse amplitude, the tymbal motoneurone was electrically stimulated to elicit a tymbal cycle, while the tensor nerve was electrically stimulated at different rates from 10 to 200 Hz (Fig. 9A,B). Stimulation frequencies of the tensor nerve lower than 30 Hz had no effect on the sound-pulse amplitude nor on the time lag of the sound pulses, but at frequencies higher than 30 Hz, there was a marked reduction in the amplitude and the time lag of the IN pulses. Furthermore, the changes to the IN pulse amplitude and time lag were rather sudden with the onset of electrical tensor nerve stimulation and affected virtually the first tymbal cycle during the stimulation. The amplitude reduction of the IN pulse was approximately 9–12 dB and intermediate amplitude and time lag values of IN sound pulses were rarely observed. The OUT pulse amplitude was essentially unaffected by the tensor nerve stimulation (Fig. 9C).

A sudden change in amplitude of the IN sound pulses was

obvious both in sound recordings during the calling song (Fig. 8A) and during electrical stimulation of the tensor nerve (Fig. 9A,B). Examination of the relative timing of activity between the tymbal motoneurone and tensor nerve stimulation revealed that there was a time window of approximately 20 ms within which a single electrical stimulus applied to the tensor nerve could elicit complete modulation of the IN pulse amplitude (Fig. 10). At the beginning and end of that time window, tensor contraction was not always effective (Fig. 10).

The mechanical properties of the tymbal of *Tymp. gastrica* appear to be similar to those of *Tett. josei* and *Tett. argentata*: quiet pulses are generated by a flattened tymbal, while the time lag increases when louder pulses are produced due to the increased convexity of the tymbal. However, tensor muscle action in *Tymp. gastrica* results in the opposite effect to that seen in the two *Tettigetia* species: contraction of the tensor muscle induces a reduction in amplitude and time lag rather than an increase. Consequently, if tensor muscle contraction induces the tymbal to become more flattened for the production of quiet sound pulses, less force should be required for the tymbal muscle to buckle the tymbal. Without electrical stimulation of the tensor muscle, a force of approximately 0.18 mN was required to buckle the tymbal in *Tymp. gastrica*. The force decreased only slightly but significantly to approximately 0.15 mN when the tensor muscle was contracted (mean from two males, standard deviation was less than 0.01 mN). Visual observation during these force measurements confirmed that the tymbal always buckled completely.

Morphological examination of the tymbal tensor system in all three species revealed a comparatively large tensor sclerite surrounded by membranous tissue which allowed large movements (Fig. 2A,C). In order to describe the morphological changes induced by contraction of the tensor muscle on the tymbal, we observed the tymbal while contraction of the tensor muscle was induced (Fig. 11). For *Tett. josei* (Fig. 11A), the largest movement was observed at the tensor sclerite (inwards), with smaller movements of the tymbal plate (outwards), the ventral rib area (outwards) and the

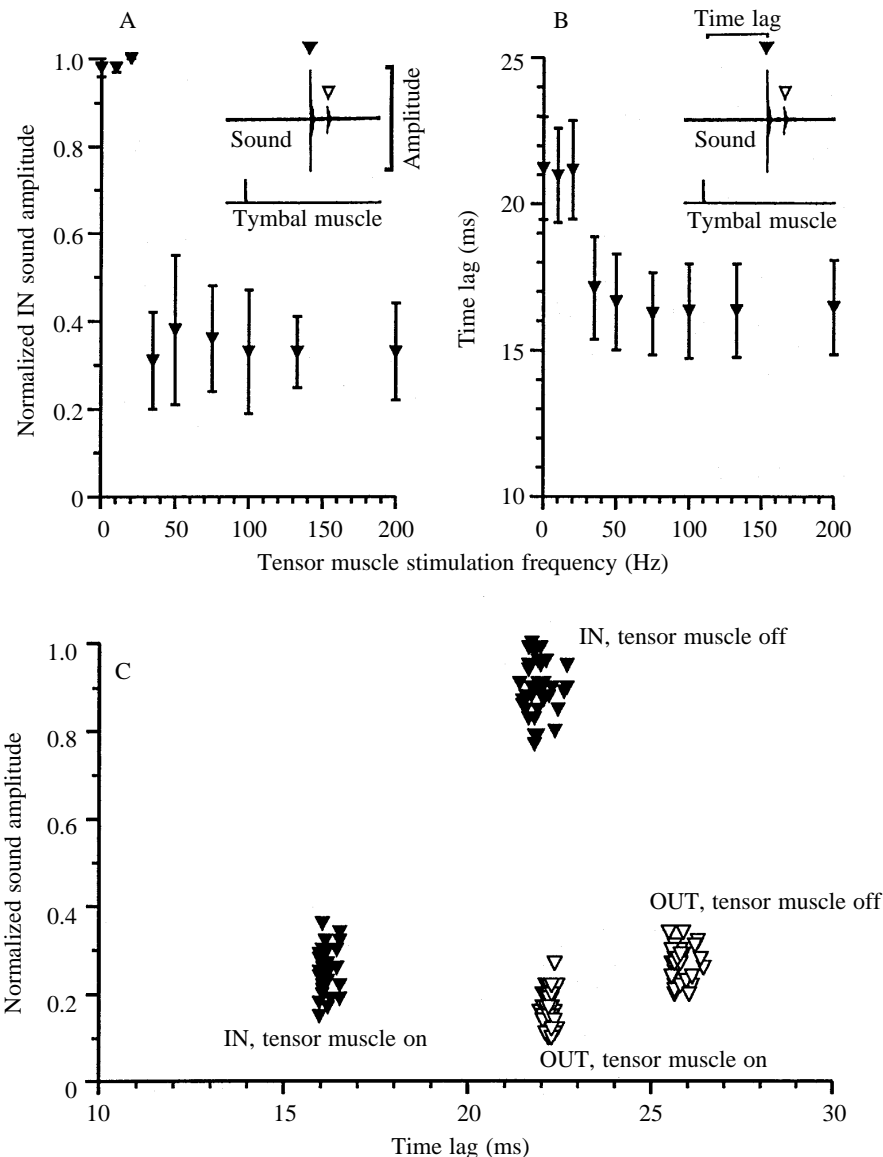


Fig. 9. Effects of electrical stimulation of the tymbal motoneurone of *Tympanistalna gastrica* (20 Hz) and concomitant electrical stimulation of the tensor nerve at frequencies up to 200 Hz. Insets show sound recordings of a tymbal cycle with electrical stimulation of the tensor nerve and illustrate how sound amplitude and time lag were determined for IN sound pulses (filled symbols). OUT, open symbols. (A) There was a sudden reduction of the IN sound pulse amplitude at stimulation frequencies higher than 30 Hz. (B) The time lag from stimulation of the tymbal motoneurone to the IN sound pulses decreased sharply at stimulation frequencies higher than 30 Hz (mean values \pm s.d. from five males). (C) Relationship between amplitude and time lag from electrical stimulation of the tymbal motoneurone for IN and OUT pulses from a tensor nerve stimulation series. Amplitudes are normalized to the amplitude of the loudest IN pulse. The amplitude and time lag values decreased with electrical stimulation of the tensor nerve. IN (filled symbols), OUT (open symbols); data from one male.

dorsal rib area (inwards). The posterior medial rib area moved outwards, whereas the anterior medial rib area moved inwards. The schematic cross section of the tymbal summarizes these observations (Fig. 11A).

In *Tymp. gastrica* (Fig. 11B), the largest movement was observed for the tensor sclerite, which moved inwards, especially at its dorsal posterior edge where the tensor muscle inserts. Other movements of the tymbal plate and the ventral and dorsal rib areas were small and directed inwards (Fig. 11B). The ventral edge of the tensor sclerite is connected to the tymbal frame and therefore the ventral mobility is reduced. Contraction of the tensor muscle will pull the tensor sclerite inwards, but also push down the ventral part of the tymbal frame (Fig. 11B). The mechanical construction of the tymbal frame is bistable and will only change if the force exerted by the tensor muscle *via* its sclerite is above a certain mechanical threshold. This particular mechanical construction of the tymbal frame reverses the 'normal' action of the tensor

muscle and, at the same time, introduces a bistability to the frame, which reduces the likelihood of intermediate amplitudes of IN sound pulses.

Discussion

The tensor muscle has a pivotal role in the production of the species-specific signal of the cicada species investigated. The fast and large amplitude modulations of the calling songs are achieved firstly by a phasic component in the action of the tensor muscle and secondly by the buckling mechanism of the tymbal and by the mechanical construction of the tymbal frame, which allow fast modulations of the shape of the tymbal.

Principal action of the tensor muscle

The tymbal muscle contracts, causing the tymbal to buckle. This produces sound, and the tensor muscle modulates the sound amplitude by changing the tension of the tymbal *via* the

tymbal frame. Hence, the shape of the tymbal relates to the force that is required to buckle it and, *via* the force produced by the tymbal muscle, also relates to the amplitude and time lag of the sound pulse. Thus, it is the construction of the tymbal frame which determines the effect of tensor muscle

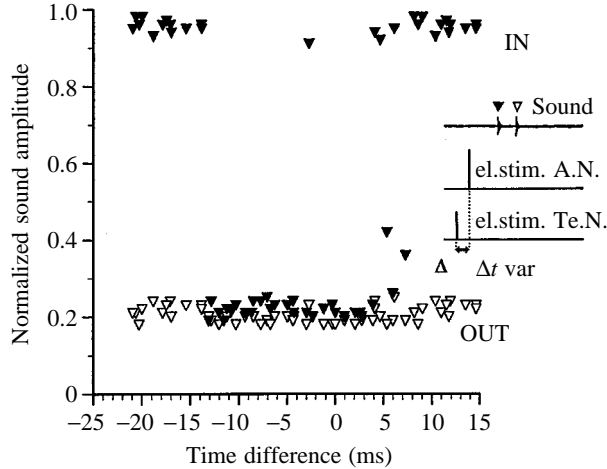


Fig. 10. Dependence of the amplitude of IN sound pulses on the relative timing of tymbal motoneurone (A.N.) and tensor nerve (Te.N.) stimulation (el.stim.) in *Tympanistalna gastrica*. Electrical stimulation of the tensor nerve from 15 ms before to 5 ms after tymbal motoneurone stimulation abruptly changed the sound pulse amplitude. Amplitudes are normalized to the amplitude of the loudest IN pulse. IN, filled symbols; OUT, open symbols; data from two males. The inset defines the time difference (Δt var) between the two stimulus pulses.

contraction: an increase in convexity in *Tett. josei* and *Tett. argentata*, but a decrease in *Tymp. gastrica*. Such opposite effects of tensor muscle action have been reported previously for other species (an increase in amplitude by Pringle, 1954a; Hagiwara, 1956; Simmons and Young, 1978; a decrease in amplitude by Weber *et al.* 1988; Hennig *et al.* 1994). In these cases, however, the mechanism is likely to be less dependent on the construction of the tymbal frame. The range of amplitude modulation described previously differs greatly from the effects observed here: less than 6 dB (Pringle, 1954a; Hagiwara, 1956; Simmons and Young, 1978; Weber *et al.* 1988; Hennig *et al.* 1994) compared with 10–20 dB in the present study. It is quite conceivable that these differences are related to the buckling mechanism of the tymbal: Popov (1975) distinguished tymbals with fewer than four ribs which buckle virtually synchronously to produce one IN and one OUT sound pulse and tymbals with more than four ribs which buckle sequentially so that each rib produces one sound pulse with the OUT pulse being quite quiet. Intermediate types where the ribs buckle sequentially, but with a very short delay, such that the pulses fuse also occur. It appears that only tymbals of the first type show a strong amplitude modulation caused by the tensor muscle and that this modulatory range may also be related to the stiffness of the tymbal itself. Tymbals which produce one IN and one OUT sound pulse are considered to be rather stiff (see Fig. 6; Popov, 1975). However, force measurements showed that the tymbal of *Tymp. gastrica* is quite soft. As yet, it is not possible to compare the results of the force measurements of the tymbal as a measure of its stiffness, but comparative research directed towards both a morphological

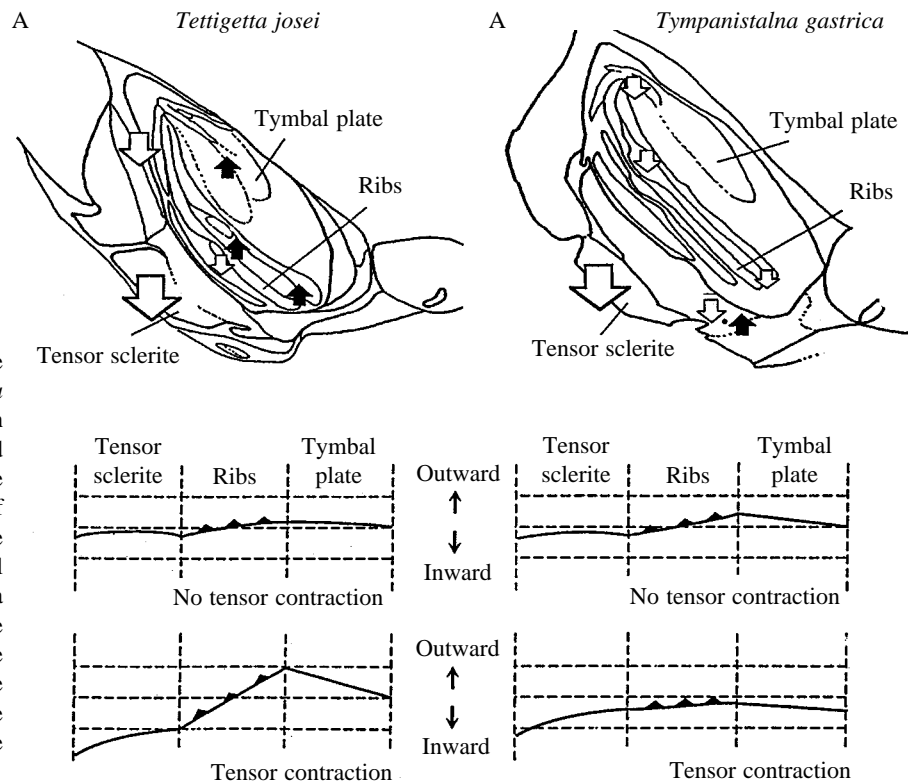


Fig. 11. Perspectively flattened view of the tymbal of *Tettigetia josei* (A) and *Tympanistalna gastrica* (B) and the areas on the tymbal which show inward (open arrows) or outward (filled arrows) movements resulting from the contraction of the tensor muscle. The anterior of the male is to the left. In *Tymp. gastrica*, there was also a joint-like movement in the tymbal frame ventral to the tensor sclerite indicated by a dot and two arrows pointing in opposite directions. Schematic cross sections through the medial region of a tymbal (perpendicular to the ribs) are presented below the tymbals. The observed movements due to contraction of the tensor muscle are shown.

and a functional description of the tymbal will certainly allow a much better understanding of the principles and variations of sound production in cicadas.

Speed of action by the tensor muscle

The phasic contraction and fast effect of the tensor muscle observed here are in sharp contrast to the known tonic action of the tensor muscle (Pringle, 1954a; Hagiwara, 1956; Simmons and Young, 1978; Weber *et al.* 1988; Hennig *et al.* 1994). However, the action of the tensor muscle described here is not only phasic but also tonic for two reasons. First, the phasic action accounts for only 30% of the amplitude modulation in both *Tettigetia* species. In order to obtain the full range of amplitude modulation in these species, the tensor muscle had to be activated at frequencies higher than 50 Hz. Second, even though the tensor muscle action is very fast, values of intermediate amplitude are observed both in our stimulation experiments and in the calling song of the *Tettigetia* species (Figs 4, 5, 7; Fonseca, 1991). In *Tympanistalna gastrica*, the mechanical construction of the tymbal frame enhances the fast effect of tensor muscle action. An important prerequisite for such a fast action [10–20 ms compared with 0.5 s (Hennig *et al.* 1994) to 2 s (Simmons and Young, 1978)] seems to be the increased mobility of the tensor sclerite due to the soft membrane (Fig. 2A,C). However, there are other modifications necessary for such an effect: most importantly, the timing of tensor muscle activity with respect to tymbal muscle activity becomes crucial in order to produce the species-specific signal. Notably, the change in time lag of the sound pulse due to the change in the convexity of the tymbal is rather large, i.e. approximately 30% of the contraction period of one tymbal muscle during the calling song (*Tett. josei*, Figs 3, 4; *Tymp. gastrica*, Figs 8, 9). While in many cicadas the tensor muscle enables the male to increase the sound level of the mate-attracting signal in an unspecific manner, tensor muscle activity in *Tettigetia* and *Tympanistalna* species contributes much to the species-specificity of the signal. Besides the peripheral modifications in the morphology and mechanical properties of the tymbal and tymbal frame, changes in muscle physiology from a slow- to a fast-contracting muscle and changes in the fine tuning of central nervous coordination, must have taken an important place in generating the specific calling song of these cicadas.

We thank F. Huber for his support of this work and for his comments on the manuscript. We would like to thank T. Weber and T. E. Moore for stimulating discussions about our data, B.

Ronacher for critically reading the manuscript and H. Bamberg for technical support. We also thank two anonymous referees for their critical comments on an earlier version of the manuscript. We gratefully acknowledge Brüel and Kjaer Portugal for the loan of equipment for measuring the acoustic conditions of our apparatus.

References

- BENNET-CLARK, H. C. AND YOUNG, D. (1992). A model of the mechanism of sound production in cicadas. *J. exp. Biol.* **173**, 123–153.
- BOCK, C. (1987). A quick and simple method for preparing soft insect tissues for scanning electron microscopy using Carnoy and hexamethyldisilazane. *Beitr. elektronenmikr. Direktabb. Oberfl.* **20**, 209–214.
- FONSECA, P. J. (1991). Characteristics of the acoustic signals in nine species of cicadas (Homoptera, Cicadidae). *Bioacoustics* **3**, 173–192.
- FONSECA, P. J. AND POPOV, A. V. (1994). Sound radiation in a cicada: the role of different structures. *J. comp. Physiol. A* **175**, 349–361.
- HAGIWARA, S. (1956). Neuro-muscular mechanism of sound production in the cicada. *Physiol. comp. Oecol.* **4**, 142–153.
- HENNIG, R. M., WEBER, T., MOORE, T. E., HUBER, F., KLEINDIENST, H.-U. AND POPOV, A. V. (1994). Function of the tensor muscle in the cicada *Tibicen linnei*. *J. exp. Biol.* **187**, 33–44.
- MOORE, T. E. AND SAWYER, R. T. (1966). The mechanism of cicada tymbal action (Insecta: Homoptera: Cicadidae). *Am. Zool.* **6**, 509.
- POPOV, A. V. (1975). The structure of the tymbals and the characteristics of the sound signals in singing cicadas (Homoptera, Cicadidae) in the southern regions of the USSR. *Ent. obozr.* **54**, 258–291.
- PRINGLE, J. W. S. (1954a). A physiological analysis of cicada song. *J. exp. Biol.* **31**, 525–560.
- PRINGLE, J. W. S. (1954b). The mechanism of the myogenic rhythm of certain insect striated muscles. *J. Physiol., Lond.* **124**, 269–291.
- REID, K. H. (1971). Periodical cicada: mechanism of sound production. *Science* **172**, 949–951.
- SIMMONS, P. AND YOUNG, D. (1978). The tymbal mechanism and song patterns of the bladder cicada, *Cystosoma saundersii*. *J. exp. Biol.* **76**, 27–45.
- WEBER, T., MOORE, T. E., HUBER, F. AND KLEIN, U. (1988). Sound production in periodical cicadas (Homoptera: Cicadidae: *Magicicada septendecim*, *M. cassini*). In *Proceedings of the Sixth Auchen Meeting, Turin, Italy, 7–11 September 1987*, pp. 329–336.
- YOUNG, D. (1975). Chordotonal organs associated with the sound producing apparatus of cicadas (Insecta, Homoptera). *Z. morph. Tiere* **81**, 111–135.
- YOUNG, D. AND BENNET-CLARK, H. C. (1995). The role of the tymbal in cicada sound production. *J. exp. Biol.* **198**, 1001–1019.