Acoustic Communication in Cicadas (Homoptera, Cicadoidea): Sound Production and Sound Reception

Thesis · January 1994

DOI: 10.13140/RG.2.1.3510.9289

1 author:

P.J. Fonseca
University of Lisbon
137 PUBLICATIONS **1,870** CITATIONS

SEE PROFILE

Paulo Jorge Quintais Cancela da Fonseca

Teaching Assistant at the "Faculdade de Ciências de Lisboa" (Portugal)

Acoustic Communication in Cicadas (Homoptera, Cicadoidea): Sound production and sound reception

Lisboa - 1994

Paulo Jorge Quintais Cancela da Fonseca

Teaching Assistant at the "Faculdade de Ciências de Lisboa" (Portugal)

Acoustic Communication in Cicadas (Homoptera, Cicadoidea): Sound production and sound reception

A thesis submitted to the Faculty of Sciences, University of Lisbon, Portugal

Lisboa - April 1994

This thesis is dedicated to my wife (Conceiçäo) and kids (Ana, Pedro and João), who gave me all their love and support throughout the ups and downs of such a long work.

Index

Acoustic communication in cicadas

Sumário

A observação do comportamento das cigarras (Homoptera, Cicadicae) no seu ambiente natural mostra que a utilização de sinais sonoros tem um papel determinante na comunicação destes insectos. Esta tese investiga a produção e recepção de som em várias espécies de cigarras que ocorrem em Portugal. Esta informação foi utilizada na perspectiva da compreensão das diferentes tarefas a desempenhar pelos intervenientes num sistema de comunicação acústica. Enquanto o emissor tem que produzir sinais sonoros detectáveis e específicos, os problemas para o insecto receptor são detectar os sinais, localizar a fonte sonora e reconhecer a sua mensagem.

A diversidade e a especificidade dos sinais sonoros, quer no seu padrão temporal e de amplitude, quer no domínio da frequência, foram ilustrados com várias espécies de cigarras: Cicada barbara lusitanica, C. orni, Tettigetta argentata/atra, Tett. estrellae, Tett. josei, Tettigetta sp., Tibicina quadrisignata e Tympanistalna gastrica. A morfologia dos tímbalos bem como de outras estruturas relacionadas com a produção e a recepção de som foi também estudada.

O padrão temporal das contrações do músculo do tímbalo e os pulsos sonoros gerados em cada ciclo muscular foram descritos para algumas das espécies durante a produção do sinal de chamamento (calling song) e do sinal de alarme (alarm signal). A coordenação bilateral dos tímbalos foi também apresentada. Entre as várias espécies de cigarras estudadas nesta tese foram encontradas diferenças importantes no que diz respeito a todos estes aspectos. Para além disto, este estudo também demonstrou que as contracções do músculo do tímbalo não podem, só por si, ser responsáveis pelas modulações em amplitude e frequência exibidas pelos sinais sonoros.

Foram estudados diversos mecanismos emvolvidos na modulação dos sinais sonoros: 1) O músculo tensor é utilizado para modificar a mecânica do tímbalo em várias espécies com base no mesmo princípio físico -- um tímbalo mais convexo e firme gera sinais sonoros mais

intensos quando colapsa sob a força acrescentada exercida pelo músculo do tímbalo. Contudo, em diferentes espécies de cigarras a contracção do músculo do tímbalo pode causar quer um considerável aumento da amplitude dos pulsos sonoros (Tett. argentata/atra, Tett. josei), quer uma acentuada redução na amplitude do som produzido (Tymp. gastrica). 2) Movimentos do abdomen podem modificar a radiação de som através dos tímpanos (Tib. qudrisignata) e/ou através da parede abdominal (Tymp. gastrica). Em alguns casos os movimentos do abdome podem afectar propriedades de ressonância do saco aéreo interno de origem traqueal e assim modificar a frequência do som produzido (Tib. quadrisignata).

Para a radiação de diferentes frequências do sinal de chamamento podem ser utilizadas diferentes estruturas. Em Tymp. gastrica os tímbalos säo as estruturas mais importantes na radiação do som correspondente ao pico espectral do sinal de chamamento (12-13 kHz), enquanto o abdome, de muito maior dimensäo, radia som a frequências mais baixas (cerca de 5 kHz). Os tímpanos também têm um papel na radiação do sinal de chamamento, sobretudo em componentes espectrais intermédios aos das outras duas estruturas. Alén disso, a importância relativa destas estruturas é diferente consoante se trate da radiação dos pulsos sonoros mais intensos ou dos de menor amplitude, ambos característicos do sinal de chamamento desta espécie, e cuja amplitude é determinada pela acção do sistema tensor do tímbalo. Em Tymp. gastrica o abdome näo parece funcionar como um ressonador de Helmholtz.

São discutidos os possíveis processos de modificação do som disponíveis para as cigarras produzirem sinais de chamamento com padröes distintos, bem como os diversos mecanismos empregues para emitir os sinais acústicos de forma eficiente.

As capacidades do sistema auditivo das cigarras para a detecção de som foram estudadas quer recorrendo à medição das respostas biofísicas dos tímpanos a estímulos sonoros, quer estimando os limiares de audição através de registos electrofisiológicos do nervo auditivo. Todas as espécies estudadas (C. barbara lusitanica, C. orni, Tett. argentata/atra, Tett. josei, Tib. quadrisignata e Tymp. gastrica) mostram uma sensibilidade auditiva elevada na mesma gama de frequências (3-5 kHz), independentemente dos espectros de frequência dos

respectivos sinais de chamamento. Em todas as espécies foram encontradas em determinadas gamas de frequência grandes diferenças na sensibilidade auditiva estimada com estimulação sonora ipsi- e contralateral. A direccionalidade auditiva é particularmente acentuada em frequências correspondentes ao pico espectral do sinal de chamamento coespecífico, e, à excepção dos machos de Cicada spp., também na gama de frequências correspondentes à máxima sensibilidade auditiva. A reduzida direccionalidade auditiva encontrada nos machos de C. barbara lusitanica e C. orni na gama de frequências correspondente ao máximo do espectro do sinal de chamamento é provavelmente devida a propriedades resonantes do saco aéreo do macho. As diferenças na resposta vibracional do tímpano, medidas através de vibrometria laser e com estimulação sonora ipsi- e contralateral em Tymp. gastrica e Tett. josei, podem atingir 20 dB ou serem mesmo superiores. Igualmente, nestas duas espécies foi encontrada direccionalidade auditiva correspondente codificada nas respostas do nervo auditivo.

As propriedades biofísicas das estruturas auditivas que possibilitam a direccionalidade na audição foram estudadas em pormenor na cigarra Tymp. gastrica. Foi descoberto que o sistema funciona como um receptor de diferença de pressão. Neste mecanismo o som pode chegar a ambos os lados da membrana do tímpano, possibilitando assim o estabelecimento de direccionalidade auditiva a baixas frequências nestes animais de reduzidas dimensões. Os acessos principais do som à superfície interna do tímpano e responsáveis pela direccionalidade auditiva foram identíficados em Tymp. gastrica, e são dependentes do sexo. No macho são sobretudo os tímpanos e os tímbalos enquanto na fêmea são os tímpanos e os espiráculos metatorácicos.

O processamento dos sinais acústicos pelo sistema nervoso foi estudado à periferia (no nervo auditivo) em diversas espécies, bem como ao nível do sistema nervoso central utilizando o registo intracelular da actividade de neurónios auditivos intermédios no complexo ganglionar metatorácico-abdominal (MAC). Estes registos intracelulares foram executados apenas en C. barbara lusitanica. O sistema nervoso pode copiar o padrão temporal geral emcontrado nos sinais sonoros coespecíficos. Contudo, com base na análise dos registos electrofisiológicos não parece possível a codificação temporal de detalhes rápidos tais como pulsos de som

individuais resultantes das acções dos tímbalos, a menos que estes sejam gerados a uma cadência baixa (approx. abaixo de 60 a 80 Hz). Foram encontrados neurónios intermédios que respondem a vários parâmetros dos sinais sonoros que podem ser importantes no reconhecimento dos sinais coespecíficos, como sejam a modulação em amplitude ou súbitos intervalos nos sinais acústicos.

A ocorrência da maior sensibilidade auditiva numa gama de baixas frequências comum a todas as espécies investigadas, conjuntamente com a falta de correspondência entre a sintonia da curva de limiar de audicção (i.e. a regiäo de máxima sensibilidade auditiva) e o espectro de frequências do sinal de chamamento, tal como se encontra em algumas espécies, é um problema do maior interesse. Esta falta de correspondência torna plausível a ocurrência de fortes pressöes de selecção diferentes das que conduziriäo à detecção dos sinais coespecíficos. Este problema é discutido no contexto de um sistema de comunicação acústica.

Summary

Behavioural observations show that sound plays a major role in cicada communication. This thesis investigates sound production and sound reception in several species of cicadas from Portugal as an approach to the tasks faced by the participants in an acoustic communication system. The problem for a sender is to produce detectable and specific sound signals, whereas the receiver must detect the signals, locate the sound source, and recognise the message.

The diversity and specificity of the sound signals in the time-amplitude pattern and in the frequency domain were illustrated in several cicada species: Cicada barbara lusitanica, C. orni, Tettigetta argentata/atra, Tett. estrellae, Tett. josei, Tettigetta sp, Tibicina quadrisignata and Tympanistalna gastrica. The morphology of the timbal and other structures related to sound production and sound reception was also studied.

The time pattern of the timbal muscle contraction and the sound pulses produced in each timbal cycle were described for some of those species during the generation of the calling song and the alarm signal, as well as the bilateral coordination of the timbals. Important differences among species were found concerning all these aspects. Moreover, this study also demonstrated that the contractions of the timbal muscles cannot be responsible for the amplitude and frequency modulations exhibited by the songs.

Several mechanisms by which sound modulation is achieved were studied: 1) The tensor muscle is used to modify the timbal mechanics in several species under the same physical principle -- a more convex, and thus stiffer, timbal generates louder sounds when buckled. However, in different cicadas the contraction of the tensor muscle can cause either a large increase (Tett. argentata/atra, Tett. josei) or an extensive decrease in the amplitude of the sound pulses. 2) Movements of the abdomen may modify the radiation of sound through the tympana (Tib. quadrisignata) and/or by the abdominal wall (Tymp. gastrica). In some cases the abdominal movements may affect resonant properties of the internal tracheal air sac and therefore the sound frequency (Tib. quadrisignata).

Different structures may be used for radiation of the calling song at different frequencies. In Tymp. gastrica, the timbals radiate more sound at the spectral peak of the song (12-13 kHz)

and the larger thin abdominal wall radiates sound at lower frequencies around 5 kHz, while the tympana are also radiating sound. The relative importance of these structures for sound radiation is different in the loud and soft pulses which are characteristic for the calling song and determined by the action of the tensor system. The abdomen of Tymp. gastrica does not seem to function as a Helmholtz resonator.

The possible sources of modification available to cicadas to produce distinct calling song patterns and the different mechanisms employed to broadcast the signals efficiently are discussed.

The capabilities of the hearing system of cicadas to detect sound were studied both by measuring the biophysical responses of the tympana and by estimating the auditory nerve thresholds with recordings in the whole auditory nerve. All the species studied (C. barbara lusitanica, C. orni, Tett. argentata/atra, Tett. josei, Tib. quadrisignata and Tymp. gastrica) show a high hearing sensitivity in the same frequency range (3-5 kHz), irrespective of the spectra of their calling songs. Large differences in hearing sensitivity with ipsi- and contralateral sound stimulation were found in all species at certain frequencies. Directional hearing was enhanced at frequencies corresponding to the spectral peak of the conspecific calling song as well as in the region of higher hearing sensitivity in all species except for males of Cicada spp.. In these species a reduction of directional hearing in the range of the calling song peak is probably due to resonant properties of the tracheal air sac of the male. Moreover, differences in the vibrational response of the ear measured with sound ipsi- and contralateral, could reach 20 dB or even more, and were found encoded in the auditory nerve responses in Tymp. gastrica and Tett. josei.

The biophysical properties of the ear allowing directional hearing were studied in detail in the cicada Tymp. gastrica. It was found that the ear works as a pressure difference receiver. This mechanism allows that sound can reach the tympanum from both sides and directional hearing at low frequency is established in these small animals. The main inputs to the ears were sex-specific and were identified as the tympana and also the timbals in the males and the 3rd spiracles in females.

Processing of sounds by the nervous system was studied at the periphery (auditory nerve) in several species, and centrally by recording from single auditory interneurons within the metathoracic-abdominal ganglionic complex (only in C. barbara lusitanica). The nervous system can copy the general time pattern of the songs but it seems not to be able to follow fast details such as the single timbal actions, unless they are produced at a slow rate (approx. below 60 to 80 Hz). Single cells were found responding to several parameters of the songs that may be required for recognition of the conspecific signals, such as the characteristic amplitude modulation or sound gaps.

The occurrence of best hearing sensitivity found in all species at the same low frequency range, along with the mismatch between the tuning of the auditory threshold curve and the calling song spectrum that seems to occur in some species is a most interesting problem. This mismatch likely indicates that strong selection pressures other than those for detection of the conspecific song may have occurred. This problem is discussed in the context of a sound communication system.

General introduction

In warm Summer days, when sitting out in the field and trying to push away my thoughts, I become aware of the many environmental stimuli leading me to the recognition of the very beauty of nature. If suddenly a heavy cloud obscures the sun, I realize that the acoustic background, previously dominated by cicada sounds, has changed. Now these songs became sporadic and let me pay attention to other softer sounds such as the ones generated by the nearby grasshoppers. Why is such a multitude of acoustic stimuli out there? How do many of these tiny animals, like cicadas, manage to generate such a diversity of sound signals and sometimes so loud? What are these signals for? By what mechanisms do they receive, process and recognize information within the miscellanea reaching my ears?

Some of these questions which drove me into the work making up this thesis did certainly arise a long time ago. In Ancient Greece cicadas were already known to generate sound, but this was attributed to wing knocking. Aristotle, however, already knew that only males produce the loud noises, and he was also able to locate the organ generating them (Bodson 1976). Recognition of the timbal mechanism used by the majority of male cicadas to produce sound is attributed to Reaumur (1741). It was, however, only late in this century that the basic functioning of the timbal was studied (e.g. Pringle 1954). Similarly, the ability of insects to hear sounds was doubtful until this century.

Cicadas rely on acoustic communication for successful reproduction. Male cicadas produce distinct acoustic signals in different behavioural contexts. The most common sound generated is the calling song, whose main function seems to be related with the attraction of mates from a distance. Moreover, this signal is also used in many species to congregate males in a chorus (e.g. Alexander and Moore 1962; Doolan 1981), and it may contribute to keep the insects within a restricted area or it may contribute to male spacing (Doolan 1981). When a female (or sometimes even another male) moves, flying or occasionally walking, towards the site of a calling male, it usually modifies the signal into a courtship song, that may differ in the rate and in the pattern of amplitude and frequency modulation. If it is a male which approaches, this one may itself generate a different interaction signal, which then causes the courting male to stop singing.

Finally, many species produce an usually unpatterned alarm signal when disturbed, that may be of some benefit in reducing predation (Smith and Langley 1978). Among species there is an incredible variety of songs with different time and frequency patterns, which are usually species specific, and thus allowing in many cases to distinguish otherwise morphologically very similar cicadas easily (e.g. Fonseca et. al. 1989; Boulard and Quartau 1991).

The sound signals often produced by several species singing simultaneously and mixing together in an usually complex acoustic environment are further modified in some of their features on its way to the receptor organs of a conspecific (Wiley and Richards 1978; Michelsen and Larsen 1983; Michelsen 1985; Stephen and Hartley 1991). Nevertheless the signals need to carry species-specific information that shall be perceived by the receiver. Therefore, cicadas must have mechanisms to maximize their probabilities of successful acoustic communication. These mechanisms include: a) to broadcast signals with very different spectral contents from the sympatric and synchronic species (e.g. the author's observations on *C. barbara lusitanica* and *Tett. josei*), in addition to their distinct time pattern. b) to concentrate their acoustic activities to different periods of the day, which was well demonstrated in *Magicicada* species by Alexander and Moore (1962). c) to segregate within the same common habitat. Sometimes one species concentrates in a restricted area, where it may establish a chorus or not, while a different sympatric and synchronic species may be more abundant in another nearby site. This happens for instance in *Tymp. gastrica*, a small species with a relatively soft signal. In addition, filtering mechanisms must occur at the level of the receptor's nervous system along its hearing path, and some decisive parameters transmitted to the central nervous system have to be compared and perceived by a pattern recognition system. Research in this area is still embryonic in cicadas. Another much needed research deals with the influence of the several parameters of the songs on the behavioural response of these insects (Doolan and Young 1989).

From behavioural observations it is obvious that sound plays a major role in cicada communication. The goal of this thesis is to attempt to contribute to a better understanding of the physical and physiological mechanisms in the acoustic communication system of cicadas by using several species from Portugal. Several different but complementary approaches will be

applied in the investigations of the sound production and sound reception systems. Biophysical, physiological and behavioural information will be presented throughout this work, along with some morphological and anatomical features relevant to the problems under study. Available information on these topics is relatively small in cicadas when compared to some other insects (Acrididae, Gryllidae). Sound production and sound reception will be treated in different chapters.

The chapter on Sound production will provide a comparative description of the sound producing structures and sound signals found in some Portuguese species, as well as a description of the timbal motor pattern subjacent to the song. In addition, it will investigate by which mechanisms sound modulation and radiation are achieved. This data will be used to discuss the specific tasks faced by the emitter -- the male cicada -- within the cicada acoustical communication system, i.e. detectability and specificity of the sound signals.

The chapter on Sound reception will present a comparative description of the sound receiving structures as well as some aspects of the neuroanatomy of the auditory pathway. Moreover, hearing will be studied at the periphery, looking at the mechanical response of the ears and at the summed responses in the auditory nerve to acoustic stimuli. The characteristics of the responses of some acoustic interneurons within the central nervous system will be examined as well. Similarly, these investigations will be use to discuss the tasks that have to be solved by the receiver, i.e. detection of sound, localization of the sound source, and filtering and recognition the message encoded in the sound signal in the context of a sound communication system.

Cicadas: A general approach

Cicadas are not conspicuous insects. Although their sounds are well recognized, many persons have never seen them. Cicada sounds are commonly linked to Orthoptera. Such common popular concept is well represented by the tale "La cigale et la fourmie" where la Fontaine (Poemes de Provence), made confusion with a bush cricket (Fabre 1923).

The taxonomic position of cicadas conform to the following hierarchic classification (see also Boulard 1988): Homoptera, Auchenorrhyncha, Cicadoidea, and the species included in this work belong to the families Cicadidae (genus *Cicada*) and Tibicinidae (genera *Tettigetta*, *Tympanistalna* and *Tibicina*).

Cicadas are confined to tropical and mild temperate regions (Metcalf 1963). From the 22 species of cicadas recorded in Portugal, only 13 are of certain occurrence (Boulard 1982; Quartau and Fonseca 1988; Fonseca 1991; Boulard and Quartau 1991).

The activity of adult cicadas in Portugal is restricted to the Summer time (Fig. 1), and the adults of each particular species are active at only part of this time. This makes work with living cicadas highly seasonal. Further complications arise from the patchiness of the distribution of the different species, from the great dependence of singing on the weather conditions since they will reduce very much the acoustic activity in cloudy or windy days, and from the short periods these insects can be kept alive in laboratory.

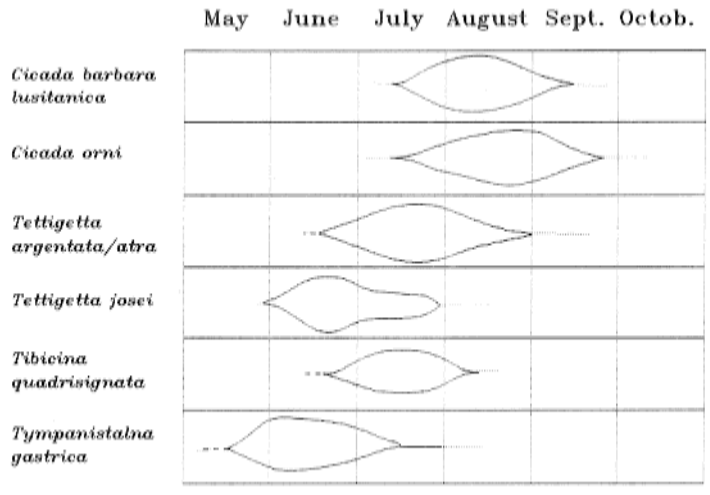


Figure 1 - Annual periods corresponding to the activities of the adults of some cicada species in Portugal, and their abundance over time. The information was obtained through several years of field work. It is, however, a subjective appreciation since no counts were made, and it is not referred to a strict place.

The characteristics of the biotops are not strict in cicadas, probably because they do not appear to be dependent on a very narrow number of feeding plants. This is likely to be linked to the xylem feeding habits found in some cicadas and possibly common to this group. Although some preferences can be recognized. For instance, species like *Tettigetta josei* and *Tympanistalna gastrica* are more common in open areas with small vegetation, while *Cicada orni* or *Tibicina quadrisignata* prefer woods or high shrubs. Other species like *Tett. argentata/atra* (cf. page 11) may be found anywhere, from coastal sand dunes with short vegetation to forests in the mountains at the interior of the country. Since these animals are not dependent of one plant species only, and because the immature stages live in the soil, where they are destroyed in mobilized agricultural fields, they are usually not considered as pests. Only when they appear in mass numbers in orchards, are they considered as an economical constraint (e.g. Banta 1960; Hamilton 1961; Smith and Linderman 1974; Karban 1982, Hogmire et. al. 1990). The feeding and ovipositing strategies used by cicadas may in principle contribute to the spread of plant diseases, but to my knowledge there are no definite studies on this subject. Natural enemies include flies belonging to the Asilidae, wasps and ants (Beamer 1928), praying mantis (the author's observations), spiders (Beamer 1928; Lockley and Young 1986; Smith et. al. 1987; Gwynne 1987), birds (e.g. Marlatt 1908; Howard 1937; Lloyd and Dybas 1966 a,b; Karban 1982, 1983; Strehl and White 1986; Steward et. al. 1988; Patterson et. al. 1991) or rodents (Wolff et. al. 1985; Krohne et. al. 1991), and all may prey upon adult cicadas which are not poisoned (see also Brown and Chippendale 1973). Underground immature stages seem to be sometimes considerably preyed by moles (Lloyd and Dybas 1966a), and are certainly a source of food for other predators living in the soil (e.g. Cumber 1952). As any other being, cicadas may present diseases and carry parasites, e.g. mites, nematodes, insects and fungi, but the studies on this subject are scarce as well (e.g. Speare 1921; Beamer 1928; Cumber 1952; Soper et. al. 1976a,b; and the author's observations).

Unlike most of other insects, cicadas present very long life cycles. Marlatt (1907) was the first to demonstrate that the 17-year Magicicada had in fact a development period taking 17 years,

with an historical experiment where he succeeded in following the development of the cicadas after transferring a large number of eggs to a wood where the species didn't occur. Other relatives need only 13 years to complete their development. Despite some species may show even longer life cycles, in the other few cases where the biological cycle was followed (e.g. Beamer 1928; Cumber 1952; Boulard 1991, 1992) these periods were much smaller and about 3 to 6 years. One reason for these very long cycles of development shall be their feeding upon xylem, which is very poor in protein and sugars (White and Strehl 1978). However, according to Slansky (1980) this in not enough to explain the extreme long life cycles found on North American periodical cicadas since other related insects, the Cercopidae, which also feed on the plants' xylem, have a more normal life span, even when corrected by their size (less than 6 months for Philaenus spumarius, Wiegert 1964). Another enigma is related to the synchronous emergencies of the adults. How do these insects emerge in the same constant period over the years, for instance in the 17 year cicadas (Alexander and Moore 1962; Lloyd and Dybas 1966a,b), that can in some cases be predicted within a short time window (Beamer 1928; the author's observations)? Moreover, how do they recognize the early hours of the day when they usually leave the ground? They cannot detect day/night length relationships based on light since the larvae live underground. Dean and Milton (1991) attributed cicada emergencies as a response to rainfall in a semi-arid region of South Africa. However, this didn't seem the cue for synchronous emergencies in tropical Panama (Wolda 1989), neither do it seem to be in some Portuguese cicadas. Apart that the emergencies seem to be more common after some rain, they occur in similar periods in rainy and dry years and even when there is no rain during the emergence period. Temperature is a possible season cue in temperate regions, and Heath (1968) suggested that synchrony in emergence might be due to animals reaching a critical threshold temperature. One other possibility is that they may detect the day/night relationship through photoperiod-induced chemical changes in the rootsap of the feeding plant (Wolda 1989).

A further mystery resides in the origin of broods, such as found in North American periodical cicadas. The origin and possible advantages of this life style leading to heavy predation during the short time where the adults are out in enormous numbers over restricted sites were discussed (e.g. Simon and Lloyd 1982; Cox and Carlton 1988).

The acoustic behaviour was only studied in a few cicada species and it was completely missing in Portuguese species, along with nearly all other aspects related to their biology. I like to illustrate my observations on the acoustic behaviour of Portuguese species with two extreme situations found in *Tibicina quadrisignata* and in *Tympanistalna gastrica*. Comparing these two species one finds some consistent similarities and differences in their acoustic behaviour. In both cases the males cease the calling song and shift to the courtship in the presence of a female, they stop singing upon contact with the female and copulation usually starts within a few seconds, keeping a side-by-side position. The so formed pair stays motionless during copulation and after that the animals move apart a few centimetres. Within some minutes the male may start calling again in the same place or may fly to another site, while the female flies out. We do not know if the female copulates more than once. There are however some important differences between the species. In *Tib. quadrisignata*, as well as in *Cicada barbara lusitanica* and other cicadas (e.g. Alexander 1960; Dunning et. al. 1979; Doolan 1981; Huber 1983), the calling male sings motionless for a long time and the female flies into the plant where the animal is signaling. The male, shifting then to courtship song, moves slowly towards the female, usually walking. The female may also walk in the direction of the male when he his close. In contrast, in *Tymp. gastrica* the male calls for a short time in a plant and then makes a short flight to start calling again in a nearby plant. Here the female seems to be already down in the plant, near the soil, and she moves not much. The male, detecting the female by sight or with any other unknown signal (for instance a vibrational signal) changes to courtship song and makes frequently very short flights accompanied by sound around the place where the female is. Finally he approaches her, and she may move towards him slowly, until contact is established and singing stopped. The calling and courtship behaviour of the Australian Cicadetta quadricincta reported by Gwynne (1987), where the female responds acoustically by wing flicking to the much mobile male, and is this one who makes phonotaxis, has many similarities with the behaviour observed here on *Tymp. gastrica*. Copulation time is much shorter in *Tymp. gastrica* (about 5 minutes) than in *Tib. quadrisignata* (up to 20 minutes or even more). In some cases the males may also produce short courtship song sequences during copulation.

Materials and methods

In this chapter I will provide detailed descriptions of the several methods used throughout this thesis. All methods are presented in a single chapter because some of them are common both to Sound production and Sound reception chapters.

1. Animals

Adult cicadas belonging to the several species studied were collected in many places mainly in central and southern Portugal during the Summer time (from May to September). The sites and periods suitable for capturing the species were recognized during previous prospecting work.

Animals for use in laboratory experiments were placed inside a cool box together with a feeding plant, transported to Lisbon University and kept on a feeding plant at 10 to 15 ^{o}C, in most cases in darkness.

Work was carried out with *Cicada barbara lusitanica*, *C. orni*, *Tettigetta argentata* (= *Tett. atra*?), *Tett. estrellae* (= *Tett. septempulsata*?), *Tett. josei*, *Tettigetta* sp. (unidentified), *Tibicina quadrisignata* and *Tympanistalna gastrica*. The identification of the *Tettigetta* species, excluding *Tett. josei*, was difficult since they are morphologically very similar. Fortunately their acoustic signals are different. Nevertheless some incertitude remain with the identification of *Tett. argentata* and *Tett. estrellae*.

Tettigetta argentata is very similar to *Tett. atra* (see Gomez-Menor 1957, and Boulard 1982). I collected samples in very different habitats throughout the country, from coastal sand dunes to mountains. Since a continuous gradation of the morphological characteristics indicated for both species was found along similar acoustic signals, and with the taxonomy of this group still unclear (Boulard, personal communication), I choose to address to these taxa as *Tett. argentata/atra* (Fonseca 1991).

A similar problem arises with *Tett. estrellae* Boulard 1982 vs *Tett. septempulsata* described by Boulard and Quartau (1991), both found in the north of Portugal. Again, both species are morphologically cryptic. The discrimination is made using the calling song. The authors base the separation of this second species on differences of the calling songs both on frequency and

on time domains. Frequency domain: The recordings of the songs on both cases were made with different equipments and nothing is said about them, their characteristics or the recording conditions; differences of this kind might create the observed disparities. Time domain: again nothing is said about the number of animals recorded nor the temperatures during the recording of the temperature dependent calling songs. Also the number of echemes in a phrase may vary. In my own observations the commonest 5-7 echemes (Fonseca 1991) characterizing *Tett. septempulsata* (Boulard and Quartau 1991) may vary as much as from 3 to 9, well superimposed to the 8 to 11 echemes indicated for *Tett. estrellae*. Also the stunted beginning of the echemes described for *Tett. estrellae* might be physiologically dependent, and there are no indications about how common was this observation. Because of the above explained incertitudes I will address to these taxa as *Tett. estrellae*.

2. Field observations

During the development of this work many hours were spent in the field, since important questions may arise from the knowledge obtained observing undisturbed animals behaving in their habitat.

Most observations were made at sight or using binoculars. In some cases a video camera was also used. In a few cases animals were tethered in the same net to allow experiments on their interactions.

3. Morphology and Anatomy of sound producing and sound receiving structures

In order to make a comparative study of the structures related to sound communication, adult cicadas were collected, killed and fixed in a solution modified after Carnoy (absolute ethanol : glacial-acetic acid : chloroform, 3:1:1) for 24 hours at room temperature (Bock 1987) and then preserved in 70% ethanol.

The observations were made with a Wild M5A stereomicroscope using whole animals as well as either longitudinal and transversal sections made with a razor blade or dissections made

with thin forceps and small scissors. The drawings were executed with a "camera lucida". Photographs and videoprints were also obtained for documentation. The photographs were made with Wild equipment while the videoprints were obtained with a Mitsubishi P70B videoprinter fed with a signal from a camera Sony DXC-101P mounted on the Wild M5A stereomicroscope. Surfaces and volumes were estimated by measurements from drawings made with the "camera lucida".

Cobalt and nickel axonal fillings of the auditory nerve towards the central nervous system were obtained adapting the methods presented by Davis (1982) and Sakai and Yamagushi (1983). Silver intensification was not used.

Basically the procedure was the following: The cicadas with wings and legs removed were waxed upside-down to a small Petri dish. The nervous system was exposed ventrally and insect Ringer was add until the animal was covered. A small cup was then built ejecting vaseline with a syringe around a small dish made with parafilm. This cup was put afloat on the Ringer and the cut end of the nerve was placed inside. Distiled water was then add to the cup and the tip of the nerve inside the water was cut. The water was removed about 1-2 minutes later and the cup filled with a 5% solution of nickel chloride or cobalt chloride. The Petri dish was covered and the preparation was allowed to rest for about 4-6 hours at room temperature or overnight in the fridge. Keeping the preparation in the fridge gave usually better fillings. After this time the cup was removed and the preparation was developed by adding some drops of a saturated solution of rubianic acid in absolute ethanol. The nickel fillings turn dark-blue while the cobalt became yellow. A mixture of the two metallic ions turns reddish, allowing for the recognition of cells with axons integrating both nerves, in preparations where bilateral fillings were made with nickel and cobalt. Nickel gave better results than cobalt with this technique because of a smaller leakage and better contrast.

The developed preparation was then washed in insect Ringer, the nervous system was dissected and fixed in a mixture of acetic acid : absolute ethanol (1:4) for about 10 minutes, and run through an ethanol series (90%, 2x 100%, 10 minutes each step). After dehydration the ganglia were cleared in methyl salicylate and viewed in wholemount preparations under a

compound light microscope. Drawings and photographs were obtained with a Leitz Dialux 20 microscope equipped with a "camera lucida" and a Wild photographic camera. The preparations could then be stored in the darkness in methyl salicylate or returned back to 70% ethanol through 4 steps (100%, 90%, 80%, 70% ethanol).

Transmission electron microscope preparations of sections of the auditory nerve were obtained by C. Bock (MPIV - Seewiesen, Germany) with the following protocol: the cicadas were ventrally dissected in insect Ringer or in fixative, which gave better results, and the portions of the nervous system were fixed during 4 h at 4 ^{o}C in 2.5% glutaraldehyde in 0.2 M cacodylate buffer pH 7.3 + 2.5% sucrose. These preparations were washed in the buffer and then dehydrated in ethanol (50%, 70%, 90%, 2x 100%), transferred to propylene oxide (2x) and embedded in Spurr. Ultrathin sections about 70 nm thick were observed in a Zeiss 10A electron microscope and pictures made in photo plates DUPONT.

Estimations of the number of auditory receptor cells were made by counting the number of axon profiles in photographs of sections obtained in the path where the auditory nerve exits the auditory organ.

4. Song recordings in the field

Sound recordings were obtained using either an AKG D202 or an UHER M518A dynamic microphone and an UHER 4000 or 4200 tape recorder at a speed of 19cm/s. This equipment has a flat frequency response up to 16 kHz (+/-3dB). The recordings were performed in the field with free and tethered animals. The distance from the microphone to the animal was always longer than one wavelength of the lowest frequency component that could be expected, to avoid near field effects (Michelsen and Nocke, 1974).

In order to study the contribution of the two timbals to the calling song, one of the timbals was carefully removed with very thin forceps along the frame of the timbal, leaving only the small region where the tendinous plate is attached. All precautions were taken to avoid damaging the timbal muscle. This operation was carried out in animals previously recorded intact. Those

animals were allowed to sing tethered over the plant where they were caught. In some species a small cut was made across the frame of the timbal (leaving the membrane as intact as possible) in order to assess the contribution of systems other than the timbal muscles to the sound production. For most of the species the results have been supported by EMG recordings on the timbal muscle (see item 7 of "Materials and methods") , simultaneously with sound recording.

As an aid to discriminate between the sound produced during the inward and the outward movements of the timbals, the timbal was driven manually applying thin forceps on the apodeme connecting the timbal muscle to the timbal, after removing the abdomen after the third segment, and the sound produced was monitored using an oscilloscope and recorded on tape. Alternatively the timbal muscle was stimulated through a pair of electrodes (minute insect pins mounted on a holder) with a triangular rectified wave. The peak voltage was high enough to obtain a maximum contraction, and the period was 80 ms, with a duration of 40 ms. To avoid any contractions due to nervous activity the nerve cord was sectioned posteriorly to the ganglia. Also the opposite timbal was removed as described before.

The acoustic signals were digitized with a PC computer equipped with a Data Translation DT2821-F-8Di, A/D, D/A converter board, at a rate of at least 50 kHz; since with the equipment used no sound components are found above 20 kHz aliasing should not play a role. In other cases the signal was low-pass filtered below 18 kHz. The data were analyzed with software developed by the author, which enables treatment in the time domain using oscillograms, and in the frequency domain, by preparing sonagrams or calculating medium power spectra using an FFT routine with a smoothing window (Parzen). The averaged spectra were calculated using at least 30 segments with either 256 or 512 active points. In order to check the quality of the programs, some of the signals were also analyzed on a Hewlett-Packard 3562A spectrum analyzer and on a Kay sonagraph. The results were similar.

5. Laser vibrometry for measurements of vibrations

All the measurements were done on the laboratory of Prof. Axel Michelsen (Odense University, Denmark). Those measurements were obtained in two different sets of experiments: 1) to study

the hearing directionality and the possible underlying mechanisms; 2) to study the vibrations of the cicada surface structures during singing and so getting hints about structures possibly involved in sound radiation. *Tympanistalna gastrica* was used in both sets of experiments.

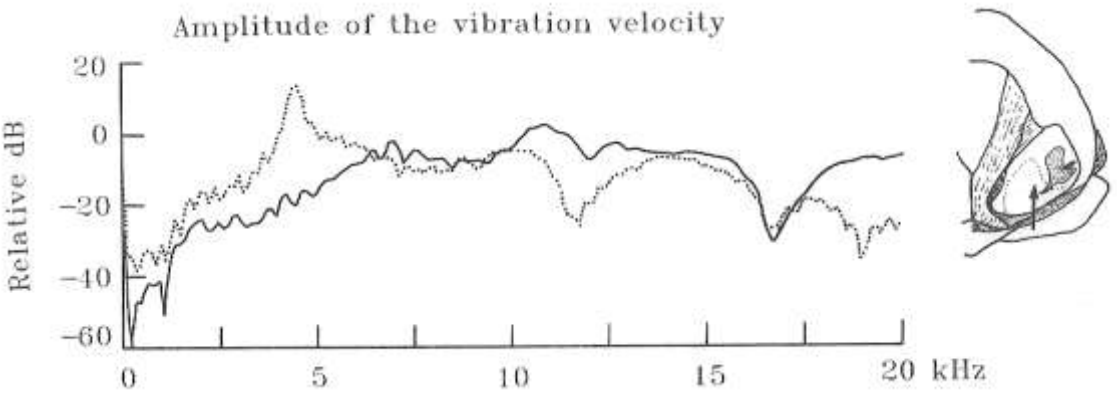


Figure 2 - Tympanic vibrations before (solid line) and after placing a small glass sphere (weighting about 0.5 microgram) on the thin tympanic membrane (dotted line), with the abdomen posterior to the 3rd segment removed. The measuring spot is indicated by the arrow on the inset drawing. The location of the peak corresponding to the large artifact caused by the sphere (dotted line) was dependent on the position of the sphere over the tympanum. The tympanic vibrations were not affected by placing the sphere over the sclerotyzed tympanic ridge.

Laser Doppler Vibrometer (LDV) measurements were obtained both with external sound stimulation and during singing (for details see Michelsen and Larsen 1978, Michelsen 1982). In most cases it was difficult or even not possible to obtain a good reflection of the laser beam from the structure selected for measurement. Therefore, a good reflecting spot for the focussed laser beam was obtained by placing a very small glass sphere (Scotchlite) on the spot to be measured. The sphere weighted about half a microgram and did not affect the vibrations of sclerotized regions. It was, however, too heavy to be used on the thin tympanic membrane, since here the sphere would create important artifacts observed on the vibrations induced on the tympanum (Fig. 2). During the directional hearing studies such a sphere was placed on the sclerotized tympanic ridge (Fig. 3). The tympanic ridge is connected to the auditory organ through the tympanic apodeme (not shown on Fig. 3) and therefore the vibrations of this region are likely to be those analysed by the auditory organ.

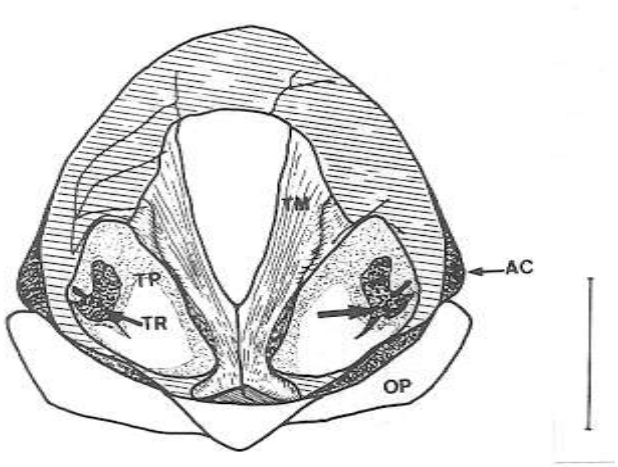


Figure 3 - Diagrammatic posterior view of the internal surfaces of the tympana of a male cicada. The abdomen posterior to the 3rd segment was removed. The thick arrow indicates the place in the sclerotyzed portion of the tympanum where the laser beam was focused at the external surface for LDV measurements. AC Auditory capsule; OP Operculum; TM Timbal muscle, TP Tympanum; TR Tympanic ridge. Scale: 2mm

In the experiments with external sound stimulation, the measured signal (vibration or sound) was fed into one of the two channels of a dual-channel spectrum analyzer (Hewlett Packard HP3562A). While the vibration signal came from the output of the LDV, the sound was measured by a probe microphone (B&K type 4182) and a measuring amplifier (B&K type 2636). The tip of the probe (1.2 mm external diameter) was as close as possible to the tympanic membrane under study and thus allowing for an estimation of the scattering of the sound waves by the body of the insect. The other channel was connected to a signal generator on the same apparatus forming the sound stimulus that, after amplification, was delivered through a loudspeaker (Dynaudio D-28 AF). The sound stimulus was formed by short burst chirps, i.e. frequency sweeps 5 ms in duration with a frequency span 0-20 kHz or 0-25 kHz, presented at 80-95 dB at the preparation (relative to 20 microPascal). Several preliminary measurements using a sequence of signals with increasing SPL allowed for a choice of suitable stimulus level.

During singing the signal from the LDV and the sound recorded by a 1/8 inch microphone (B&K type 4138) were separately fed into the two channels of a Hewlett Packard HP35665A spectrum analyzer operating as a digital recorder (time capture mode) set on a 0-25 kHz bandwidth.

The FFT-analyzers could calculate the spectra of the signals in both channels and compute the transfer function between them. The transfer functions could be corrected for the frequency responses of the speaker and the probe microphone (by comparison with a 1/8 inch microphone, B&K type 4138). In the experiments with sound stimulation, usually spectra from either 16 or 32 stimulations were averaged. The simultaneous computation of the coherence

function during the repetitions, allowed by the FFT-analyzer, was used to check for the stability of the measurement and its relation with the stimulus.

Preparation of the cicadas for the studies of directional hearing and sound scattering caused by the cicada body. The animal taken from the cold room was anaesthetised with CO2 and waxed by the mesonotum to a holder after it was turned ventral side up . The legs were removed and in some cases also the wings. The opercula were then carefully cut in order to expose the tympana. Any hole in the integument was sealed with wax. Care was taken to avoid damage of the 3rd spiracles which connect with the internal tracheal air sac and thus lead to the inner side of the tympana. Dissection of the females was much more difficult since here the opercula are thick and contain haemolymph. Thus it was necessary to remove some of the haemolymph with absorbing paper prior to sealing the cut with wax. Finally, the small reflecting sphere was positioned with the tip of a needle. Cicadas prepared this way stayed alive for several hours.

The cicada was then fixed at the end of a vertical steel rod, which was 10 cm long and had a diameter of 2 mm. This rod was attached to another rod, about 20 cm long and 6 mm across, with a fixing head that allowed the animal to be placed in a favourable position in relation to the laser beam. The arrangement was placed inside an anechoic room at the centre of a roundabout carrying a loudspeaker (Dynaudio D-28 AF), which was moved around the animal in steps of 30o. The acoustic conditions measured with the holder, but without the animal, were close to those of a free field over the frequency range studied (cf. Fig. 73 A). The distance from the speaker to the insect was 88 cm.

Reversible blockings of ears, timbals and spiracles were performed by shielding the structure with a small piece of paper and then covering it completely with wax. In order to estimate the importance of the input through the wall of the abdomen, its surface was covered with a thick layer of vaseline. To check the influence of the size and position of the abdomen on the vibrations of the tympanum, the abdomen was manipulated and held in place by a thin copper wire. The use of the reflecting sphere guarantied repositioning of the laser beam on the same spot during the experiments.

Control measurements showed that there were no vibrations of importance in the holder (<0,03 mm.s-1) in the frequency range studied.

The preparation of the animals for LDV measurements during singing was basically similar, but the arrangement was placed over an heavy table in a normal room and brain stimulation was used (see next item).

All computations were made with the FFT analyzer or with a PC computer. Calculations with dB values were made after converting to linear scale.

6. Other acoustic measurements

Other experiments based on acoustic measurements included: 1) Measurements of scattering of the sound waves by the cicada body; 2) Measurements of the sound pressure inside the abdominal posterior region of the air sac during sound stimulation; 3) Measurements of the sound radiation pattern during spontaneous calling song; 4) Study of the role of several structures on the sound radiation.

Scattering of the sound waves by the cicada body. The measurements were made with *Tymp. gastrica*, *Tett. josei* and dead males of *C. barbara lusitanica*, as well as with plasticine models of the cicadas. All measurements were conducted in the laboratory of Prof. Axel Michelsen (Odense University, Denmark).

The cicadas were mounted as indicated above for the measurements of tympanic vibrations. The sound pressure level at the centre of the roundabout was measured with a probe microphone (B&K type 4182) equipped with a 10 cm long steel probe tube (1.2 mm external diameter), and a measuring amplifier (B&K type 2636). The microphone was suspended vertically from the roof of the anechoic room fixed in a rod (approx. 1 cm in diameter and about 50 cm long) held by a micromanipulator fixed in another stronger rod descending from the roof. The micromanipulator allowed a precise centring of the microphone tip, which was obtained measuring and equalising (+/- 3 microsecond) the time elapsed between the generation of a burst

of sound by the FFT analyser (Hewlett Packard HP3562A) and its detection by the microphone. The loudspeaker (Dynaudio D-28 AF) was placed in opposite positions in two orthogonal axis.

The cicada was placed with one tympanum (usually also under LDV measurement) as close as possible to the tip of the microphone, and the measurements were made turning the loudspeaker in 30o steps. The quality of the sound field was checked with a complete series of measurements without the animal, but with the holder. This procedure allowed for an estimation of the scattering of the sound waves by the body of the insect. The sound signals were generated by the FFT analyser as indicated above, and the measurements were made also in the same way, using the transfer function calculated between the spectra of the sound stimulus and the microphone signal.

Measurements of the sound pressure inside the abdominal posterior region of the air sac during sound stimulation. In other experiments, only made with *Tymp. gastrica*, the tip of the same probe microphone, also centred in the roundabout, was inserted vertically into the abdomen and the integument was sealed around the probe with wax. This allowed for an estimation of the effect of blocking the sound input through several abdominal structures on the sound pressure inside the abdominal air sac. The values were then compared with the sound pressure measured without the animal.

I was aware that the volume inside the abdomen was too small for a proper use of probe microphones to measure the exact value of the sound pressure; however, one may still learn from the direction and magnitude of the changes in the pressure caused by experimental manipulations of the animal.

Measurements of the sound radiation pattern during spontaneous calling song. Sound radiation diagrams of the calling song of *Tymp. gastrica* were obtained in Lisbon, at the Centro de An lise e Processamento de Sinais of the Instituto Superior T,cnico. The cicadas were waxed by the mesonotum to a holder made with a steel wire 0.6 mm in diameter and allowed to walk on a styrofoam ball floating on water (see the description of the EMG recording method). The arrangement was placed inside the anechoic room. The sound measurements were made with a

1/2 inch microphone (B&K type 4134), at 100 cm from the animal. The microphone was suspended from the roof by a turning table (B&K type 3922) and turned around the animal. It was connected to an heterodyne analyser (B&K type 2010) and to a level record meter (B&K type 2307). The measurements were made in linear mode either with a highpass filter (>63 Hz) or with a 1/3 octave bandpass filter centred at 13 kHz, frequency corresponding to the spectral peak of the calling song. The animals were stimulated with light and with the conspecific calling song.

Study of the role of several structures on the radiation of the calling song. The experiments were done on the laboratory of Prof. A. Michelsen (Odense University, Denmark) in collaboration with Dr. Andrej V. Popov (Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia). The cicada used was *Tymp. gastrica*.

Preparation of the animals for measurements in the laboratory. A cicada taken from the cold room was anaesthetised with CO2. The wings and legs were cut. In some experiments the opercula were also removed with thin scissors in order to expose the tympana. The end of a steel rod (2 mm in diameter, 15 cm long) was waxed to the mesonotum of the cicada. This rod was inserted into the fixing head of another rod 6 mm across which was fixed in a vertical position on a stand placed on a heavy metal table. The movements of the head of the second rod allowed a favourable position of the animal relative to the probe microphone, which was itself mounted on a micromanipulator allowing a precise positioning.

In order to elicit singing with the normal calling song pattern we used a technique of electrical brain stimulation developed by A.V. Popov for the large cicada Paharia zevara. A pair of electrodes (minuten insect pins) were inserted into the head just medially to the compound eyes of both sides and on the line just below the ocelli. Electric pulses 2-4 V and one millisecond in duration were delivered by a stimulator through a stimulus insulation unit usually at a rate 40 to 60 Hz. For most of the animals stimulation during one or two seconds was enough to evoke singing which started within 1-2 minutes after the end of the stimulation and lasted for at least several seconds. The animals sang with a normal posture, expanding and lifting the abdomen. Since the position (shape/volume) of the abdomen might influence the sound produced, in some

of the experiments we waxed the tip of the abdomen (pygophor and hypandrium) to a thin wire which was itself fixed to the rod supporting the cicada. This procedure allowed us to keep the position of the abdomen constant throughout the experiment or to manipulate its position. The acoustics of the preparation was hardly affected by waxing the thin wire, since the tip of the abdomen is not hollow but occupied by tissues. A Wild stereomicroscope was used for preparation of the cicadas.

Sound measurements in the laboratory. The sound produced during singing was measured with a Bruel & Kjaer probe microphone (type 4182) with a 10 cm long steel probe tube (external diameter 1.2 mm). The microphone was connected to a measuring amplifier (Bruel & Kjaer type 2636) and the output signal was sent to a spectrum analyzer (Hewlett Packard HP35665A) working in time capture mode responding to 0-25 kHz. The computed spectra were corrected for the frequency response of the probe microphone (measured using a 1/8 inch microphone B&K type 4138 as reference). Measurements were obtained in 3 animals. With a micromanipulator the probe was positioned about 1-2 mm from the body surface near the timbal, the operculum or the tympanum, the ventral and dorsal mid line of the abdomen in front of the 4th segment, the tip of the abdomen and the head. In order to check the importance of the opercula, some measurements were made before and after they were removed.

Another set of microphone measurements involved manipulating the animals. In these experiments we used a 1/8 inch microphone (B&K type 4138) about 25 mm from the ventral surface of the cicada, and at a medial point of the long axis of the body. This fairly short distance was chosen in order to reduce echoes from the walls. The equipment nearby the preparation was covered by sound absorbing material. Under these conditions the echo was much smaller than the direct sound. Errors in repositioning the animal after each manipulation were reduced to a minimum by pointing a laser beam to a fixed reference area on the head of the animal. Manipulation experiments included two distinct groups: a) covering experiments and b) modifications of the internal hollow chambers.

a) Covering experiments. Microphone measurements were obtained in two different sequences of covering with vaseline different structures supposed to be important for sound

radiation. Sequence 1: 1) initial measurements on the animal with wings, legs and sometimes also the opercula removed; 2) folded membranes covered with vaseline; 3) plus tympana covered; 4) plus ventral surface of the abdomen covered; 5) plus the whole abdomen covered; 6) plus 3rd spiracles covered. In sequence 2 we covered the abdomen (2 steps) before the tympana. Three animals were used for each sequence.

b) Manipulations with the internal chambers. To study the role of the thoracic chamber we used the following procedure: 1) the song was recorded with both timbals working; 2) recording was repeated after one timbal was covered with vaseline; 3) the ribbed portion of the previously covered timbal was removed and vaseline was inserted into the thoracic chamber through a syringe mounted on a micromanipulator. The timbal was covered again and recording was repeated; 4) step 3 could be repeated after an additional amount of vaseline had been inserted into the chamber, further reducing its volume; 5) the animal was dissected to check the position of the vaseline inside the chamber. To study the role of the abdominal chamber and surface we made recordings with the abdomen maximally compressed or expanded (as during normal singing).

Spectra of the two loud clicks starting each echeme or of the four soft pulses corresponding to a complete cycle of action of both timbals were measured. In each sound measurement we usually averaged 4 spectra of clicks and 8 spectra of soft pulses.

All the computations were made with the FFT analyzers or with a personal computer. Calculations with dB values were made after converting to linear scale.

7. Electromyograms and nerve stimulation and recording

Electromyograms (EMGs) recording

The timbal muscle activity during the production of the normal calling song was recorded in *Cicada barbara lusitanica*, *Tettigetta argentata* and/or *Tett. atra*, *Tettigetta josei*, *Tibicina quadrisignata* and *Tympanistalna gastrica*. Moreover, an alarm signal was recorded in four of these: *C. barbara*

lusitanica, *Tett. argentata/atra*, *Tett. josei* and *Tib. quadrisignata*, allowing comparison with the calling song produced under the same conditions. On *C. orni*, it was only recorded an alarm signal, since I didn't succeed in evoking a normal calling song under laboratory conditions.

Preparation for EMG recording - The cicadas were anaesthetised with CO2 and waxed by the pro- and mesonotum to a holder, a metal rod 5 cm long and 2 mm across. The wings were raised and temporarily fixed to the holder or, in other cases, they were removed on the right side. The animal was then positioned under a stereomicroscope (Wild M5A) and a small window was opened on the lateral anterior right side of the 3rd abdominal tergum. The recording electrodes, made with 30 micrometer insulated steel wire, were carefully inserted through this window in one or both timbal muscles, near their insertion on the "chitinous V". The wire was then waxed to the posterior edge of the 2nd abdominal segment and to the holder. For the indifferent electrode a steel wire with the same diameter was used but the insulation was removed in the last 1-2 mm. It was inserted dorsally in the intersegmentary soft region between the 7th and the 8th abdominal segments and was fixed with wax. To allow abdominal movements the wire was looped between the attachment points in the animal and in the holder. The loop was always kept in vertical position in the medial line of the animal to avoid interference with the movements of the wings.

The holder connected to the animal was fixed to an horizontal one (10 cm long, 4 mm across) that could be adjusted in a vertical rod (50 cm long, 8 mm across) which was placed in the middle of a 1 m3 faraday cage lined with sound absorbing material (10 cm deep rockwool covered with Illbruck "super waffel"). The animal was allowed to walk on a styrofoam ball (2.5 cm in diameter) floating on a small container with water mounted in the same vertical rod. A similar method was used by Simmons and Young (1978), Josephson and Young (1979) and Young and Josephson (1983a,b).

With this arrangement (Fig. 4) the animal could walk and move its wings and was also able to move and extend the abdomen.

EMG and sound recordings - The electrodes were connected to single-ended amplifiers. The muscle potentials generated during singing were amplified 10x and monitored on an oscilloscope.

The sound produced by the animals was monitored using a Sennheiser MKE-2 microphone amplified with an UHER 4200, and was located in front of the animal, approximately 10 cm away. This distance was kept short since what was important in this study were the time correlations and not the spectral characteristics of the signals. This audio configuration was within a 5 dB variation measured in the range 0.5-20 kHz and with a relatively flat frequency response curve. The EMG and sound signals were simultaneously digitized with an A/D converter computer board (Data translation DT2821F-8DI) at rates ranging 25-30 kHz/channel recorded. The data was recorded in the computer memory and later it was stored using a tape streamer.

To reduce the echoes, all hard surfaces were covered with sound absorbing material, and the holders and microphone were kept as small as possible.

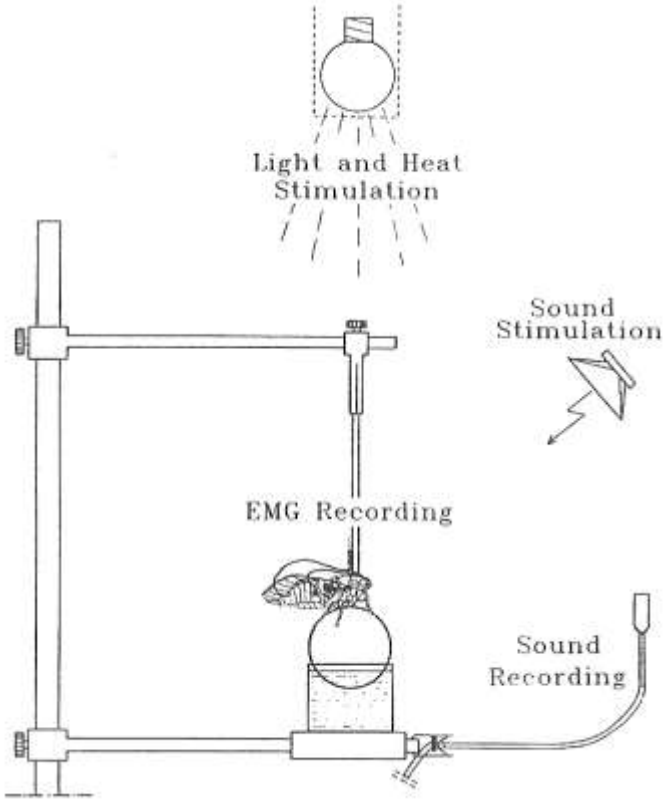


Figure 4 - Schematic diagram of the arrangement used to record the sound signals and the timbal muscles EMG from the male cicadas.

Stimulation of the animals - In order to obtain spontaneous calling songs the cicadas were kept under a lamp and stimulated by play-backs of their conspecific songs. The loudspeaker (Motorola

KSN6005a) and the lamp were placed about 40 cm away from the animal. The alarm signals were elicited by touching the animals usually at the head.

Auditory nerve recordings to evaluate the auditory thresholds and during singing

The auditory nerve thresholds were obtained in six species of cicadas: *C. barbara lusitanica*, *C. orni*, *Tett. argentata/atra*, *Tett. josei* (only males), *Tib. quadrisignata* and *Tymp. gastrica*.

Dissection of the animals. The animals were anaesthetized with CO2 and waxed to a holder similar to the one described above. The animal was placed ventral side up and the legs were removed. The metacoxae were removed and the anterior part of the bridge of integument (sternellum) between them was cut through. Thereafter the sternum posterior to the metacoxae and between the opercula was cut, and this portion was carefully lifted and removed. During the dissection the preparation was kept wet by applying drops of Ringer solution when needed. Such a preparation remained in good condition for more than two hours.

Nerve recording. The recording electrode was a single hook made of an electrolytically sharpened tungsten wire. A silver wire reference electrode was placed in a pool of Ringer and haemolymph near the measuring electrode, which was then lifted to the surface and surrounded by vaseline to increase the signal-to-noise ratio. The signal was fed to a home made single ended amplifier (1000x amplification), and monitored both with an oscilloscope and with headphones. Samples of recordings were digitized with a Data Translation (DT2821F-8DI) computer board and transferred later on to a tape streamer.

Sound stimuli and threshold estimation. The sound stimulus consisted of pure tone pulses of about 40 ms duration, shaped both at the beginning and at the end by an 8 ms ramp. The stimulus, monitored with an oscilloscope, was presented through a piezoelectric speaker (Motorola KSN6005a). The frequency response curve of the complete audio chain of equipment was later measured on the anechoic room of the Centro de An lise e Processamento de Sinais (IST,

Lisboa), using a calibrated 1/4 inch microphone (B&K type 4135) and a measuring amplifier (B&K type 2010), keeping the distance speaker-to-microphone similar to the distance speaker-to-cicada used during the experiments.

Auditory thresholds in the range 1.5-2 kHz to 40 kHz were estimated with the headphone monitoring method and an oscilloscope. Two threshold curves were usually obtained with the speaker in ipsilateral and contralateral positions by rotating the stand supporting the speaker. The experimental thresholds were later corrected for the frequency response of the audio equipment used for stimulation. Unfortunately, I did not have sound measuring equipment during the experiments. However, measurements with a microphone (Sennheiser MKE-2) and an oscilloscope showed that the echoes at the place where the animal was held were at least 30 dB below the sound stimulus.

The measurements were carried out with the preparation inside a 1 m3 sound insulated cage, lined with 10 cm thick rockwool and another acoustic absorbing material (Illbruck special waffel). The preparation was placed in the centre of the cage and 30 cm away from the loudspeaker. Insulation from the external sounds[1] was better than 50 dB for frequencies above 2 kHz, and at least 30 dB for lower frequency sounds.

Auditory nerve recordings during singing. Examples of auditory nerve activity during singing were recorded using the computer and the animals were stimulated to sing as indicated above for the EMG recordings.

The data was analysed with a PC computer and home made software. Computations on dB values were made after converting to linear scale.

Tensor nerve (TE) and auditory nerve (AN) recording and stimulation to study the modifications on the sound caused by the timbal tensor muscle, and by changes on the abdominal position

[1] Measurements were made with a sound intensity microphone pair (B&K type 4183) calibrated for SPL measurements and a sound intensity analyzer (B&K type 4433).

The experiments were done in Lisbon in collaboration with Dr. Mathias Hennig (MPIV, Seewiesen, Germany). The cicada species used were *C. barbara lusitanica*, *Tett. argentata/atra*, *Tett. josei*, *Tib. quadrisignata* and *Tymp. gastrica*.

Preparation of the cicadas for nerve recording and stimulation - The male cicada, with wings, legs and mouthparts removed, was waxed to a rod similar to the one described above. This rod was then screwed in a thicker one (6 cm long and 8 mm in diameter), which was fixed in a magnetic stand. A couple of electrodes for brain stimulation were implanted (see description above under Study of the role of several structures on the radiation of the calling song, page 24). The magnetic stand was fixed at the centre of the iron plate in the bottom of the set-up surrounded by a Faraday cage. The animals were then basically prepared as indicated above for estimation of the auditory nerve thresholds. Anaesthesia with CO2 was not used. Additionally, here the mesosternum was removed to expose the nerves until close to the metathoracic abdominal ganglionic complex (MAC), which was nevertheless not exposed. For some animals the abdominal position was controlled with a thin wire waxed between the holder and the tip of the abdomen (hypandrium and pygophor). Concomitantly, in some animals the abdominal nerves were sectioned to further reduce abdominal movements that might change the sound produced. The dissected region was flooded with insect Ringer and the tensor nerve (TE) and auditory nerve (AN)[2] of one side were lifted on double silver hook electrodes (50 micrometer in diameter) for recording and electrical stimulation. The nerves on the hooks were moved apart to exclude Ringer bridges and reduce the risk of electrical cross-talk. The Ringer was then removed and the nerves were insulated with a mixture of vaseline and mineral oil. Finally the cicada was grounded by a wire inserted into the mesothoracic leg stump. The preparation was kept moistened with Ringer, and was often viable for several hours (up to 6 hours) for recording and stimulation.

In the experiments with mechanical measurements the holder was tilted such that the timbal could be observed through the microscope. Hooking the nerves in these cases was much more difficult.

[2] The auditory nerve carries both the auditory receptor fibers and the timbal motoneuron in all cicadas used, with the exception of *C. barbara lusitanica* where a timbal nerve splits from the auditory nerve soon after the MAC. In this case, and according to the experiment, we needed to hook either the AN or the timbal nerve.

All dissections were performed under a Wild M5A stereomicroscope mounted on a movable arm in the set-up. The microscope was moved out during the sound recordings.

Sound monitoring and improvement of the acoustical conditions. In all experiments the region around the animal was covered with sound absorbing and acoustic insulation material (cotton wool and Illbruck "super waffel" pieces) to reduce the echoes coming from many hard surfaces close to the animal, such as the iron plate, micromanipulators, magnetic stands and holders. The sound generated by the cicada was monitored with a microphone (Sennheiser MKE-2) placed about 10 cm away from the animal and amplified with an UHER 4200 (see frequency response characteristics above, page). The acoustic signal was carefully examined at the beginning of each experiment and, when needed, the sound absorbing material was repositioned for further reducing echoes.

Signal recording. The signals -- sound, nerve activity and electrical stimulation signals -- were stored on tape with a DAT recorder (TEAC 100T) responding up to 20 kHz while recording on two channels or to 10 kHz using the four channels recording mode.

The experiments performed included: 1) Recording of AN and TE activities during singing elicited by electrical brain stimulation; 2) Sound recording during the electrical stimulation of the AN and TE, with and without cutting the nerves; 3) Study of the effects caused by the posture of the abdomen (size and shape) on the sound generated (frequency spectrum and damping) during electrical stimulation of the AN. 4) Experiments to study the mechanical action of the tensor muscle including: a) Electrical stimulation of the TE to observe the changes induced on the timbal and timbal frame; b) Mechanical mimicking of the tensor muscle action by pushing on the tensor sclerite both during AN stimulation and singing induced by brain stimulation; c) Measurements of the force necessary to buckle the timbal inward both with and without TE stimulation.

Recording of AN and TE activities during singing elicited by electrical brain stimulation. Singing was elicited with brain stimulation as described above. In many preparations the normal calling

song pattern was obtained as an after-effect. Especially in such cases, the activity in the auditory and tensor nerves during singing resulted in clear recordings, since there were no electrical artifacts caused by the stimulation.

The differentially recorded nerve signals were amplified 500x-1000x, low-pass filtered below 10 kHz and stored on tape using two channels of the DAT recorder. The other two channels received the time characteristics of the stimulus and the microphone signal. All signals were monitored on oscilloscope.

In some males one or both TE were lesioned to observe the respective effects on the normal calling song pattern. Sometimes this was made in animals singing only with one timbal.

Sound recording during the electrical stimulation of the AN and TE. The nerve stimuli, formed in pulse-train generators, were delivered to the preparation through insulated battery units. The duration of the pulses was 0.5 ms and the frequencies used ranged from 5 Hz to 50 Hz (rarely 100 Hz) for the AN (timbal motoneuron) and 10 Hz to 200 Hz for the TE. The amplitude, set in the stimulus insulating unit, was adjusted in order to elicit a response from the muscles. Usually a longer AN stimulus train-of-pulses triggered, using a delay unit, a shorter TE train that occurred in the middle of the AN train, thus allowing an easier observation of any effects caused by the tensor stimulation on the sound produced. Each stimulus was run through a switch which allowed an independent control of the delivery of the trains. If switched off no signal would be monitored.

The signals, nerves stimuli and sound, were monitored with an oscilloscope and stored with the DAT recorder set to two channels recording. AN and TE stimuli were superimposed and so recorded in the same channel. Discrimination was allowed by a different attenuation of both stimuli.

An experiment might include a) the variation of the rates of TE stimulation (from 10 to 200 Hz) under several fixed rates of AN stimulation (5 Hz, 20 Hz, 50 Hz); b) the variation of the AN stimulation rate keeping the TE stimulation constant (e.g. 10 Hz), and also the search for the maximum possible rate; c) repetition of some series of a) after cutting the nerves next to the MAC, to check for differences that could be related to any feed-back on the CNS.

In some male cicadas a mixed experiment was conducted where the TE (intact or cut) was stimulated during the calling song elicited by brain stimulation, and so inducing the respective effects caused by the contraction of the tensor muscle.

Study of the effects caused by the position of the abdomen (size and shape) on the sound generated (frequency spectrum and damping) during electrical stimulation of the timbal motoneuron. These experiments were done with *C. barbara lusitanica* and *Tib. quadrisignata*, since these species show relatively slow song modulations, in principle compatible with modifications in the abdominal position. Moreover, a correlation between abdominal position and amplitude modulation is readily observed in singing males of *Tib. quadrisignata* (Fonseca 1991). To monitor the modifications in the sound (frequency spectra and damping) caused by changing the posture of the abdomen, the abdominal position was varied during electrical stimulation of the AN (*Tib. quadrisignata*) or timbal nerve (*C. barbara lusitanica*). A micromanipulator with a rod (20 cm long, 4 mm diameter) fixing a wire waxed to the tip of the abdomen (hypandrium and pygophor) allowed for the control of the abdominal position. Nine positions were selected: maximum compressed, medial and maximum extended abdominal lengths held far upward, medial or far downward. The extreme positions do not seem to occur in a normally behaving animal, but they were a simple reference to our experiment. The abdominal nerves were cut to prevent active movements by the animal. For all positions the induced sound pulses were stored in the DAT recorder set to 2 channels recording. The frequency of the nerve stimulus was selected to induce sound pulses as close as possible to the calling song ones and it was kept constant during the series.

Observation of the changes induced on the timbal and timbal frame during electrical stimulation of the TE. The tensor muscle contraction was induced by TE stimulation, usually at about 100 Hz. The resulting morphological changes, observed with a stereomicroscope (WILD M5A), were annotated on a drawing of the timbal previously made with a "camera lucida", together with remarks about the direction of movement (inward or outward) as well as the strength of the displacement (noted in arbitrary indication of small, medium or large). Movements in the plane of

the timbal were indicated using arrows (e.g. movement of the ribs relative to each other). Movements around a joint were also noted.

The observations were done by one of us and then confirmed by the other.

Mechanical mimicking of the tensor muscle action by pushing on the tensor sclerite. Pushing the tensor sclerite during sound generation was done with a tipless insect needle inserted in a holder (20 cm long) which was mounted on a micromanipulator. The needle was positioned in such a way that it would mimic the tensor sclerite movement during the tensor muscle contraction. The tip of the needle, at first just touching the sclerite, was advanced by steps measured in the micromanipulator scale, while recording the sound. Sound was elicited by AN (timbal motoneuron) stimulation and, in some cases, the experiments were also done during singing induced by brain stimulation.

Measurements of the force necessary to buckle the timbal inward both with and without TE stimulation. The timbal was loaded with force exerted by a spring mounted on a micromanipulator, with and without an electrically induced tensor muscle contraction. The tip of the spring, only 100 micrometer in diameter, was placed close to the insertion point of the timbal muscle apodeme. The force was increased steadily by advancing the micromanipulator until buckling of the timbal was obtained, usually accompanied by sound. The force needed was then determined from the micromanipulator reading, which was compared with a calibration curve made with a balance.

Such a small tip of the spring, the place over the timbal where the force was applied and the angle of incidence of this force, which was selected to approach what we thought that should be the timbal muscle one, proved to be critical for successful measurements. Applying the force in other places on the timbal plate or modifying the angle usually resulted in no measurable differences between the force applied before and after tensor contraction.

Once everything was correctly placed, the measurements were taken, two for each contraction condition induced by electrical stimulation of the tensor nerve. The series of stimulation frequencies was selected according to the ones used before. Each of us did a series

of measurements in the same timbal and the average was taken as a result. The measurements were made such that the person pushing the spring and controlling visually the timbal buckling had very little idea about the readings and stimulation frequencies used.

Data analysis was made with a MACINTOSH computer equipped with appropriate software (MacRecorder and SuperScope). Chart prints were made with a 8 channel chart recorder (PICKER UNISCRIPT UD210), after transferring the data from the DAT recorder to a RACAL Store 4DS at 7 1/2 inch.s-1 and playing it to the chart recorder at 15/16 inch.s-1.

8. Single cell recordings

Recordings from single acoustic interneurons of *C. barbara lusitanica* were done on the laboratory of Professor Franz Huber (Max-Planck-Institute fur Verhaltensphysiologie, Seewiesen, Germany).

Preparation of the cicadas. The wings, legs and mouthparts of a cicada taken from the cold room were removed. The insect was then turned ventral side up and waxed along all its dorsal region to a holder made of a plexiglass block mounted on a magnetic stand. Care was taken to avoid waxing the timbals. The Metathoracic abdominal ganglionic complex (MAC) was exposed ventrally under a WILD stereomicroscope and the dissected region was flooded with insect Ringer. The nerves to the strong wing muscles were cut, as well as most nerves exiting the MAC complex, to reduce movements during the recording. Care was taken to avoid cutting the auditory, tensor and abdominal nerves, as well as to prevent damage of the metathoracic spiracles, timbals, folded membranes and abdomen.

Enzymes were used to soften the sheath surrounding the MAC and so allowing an easier electrode penetration. The Ringer was removed and a small amount of either protease or collagenase was allowed to act during 30 to 60 s. The enzyme was several times washed with Ringer.

The preparation was then transferred to the vibration insulated set-up where a spoon, made from a shaped syringe needle and supported by a micromanipulator, was carefully placed under the ganglionic complex from the posterior side. The spoon was then slightly lifted and so it helped to stabilize the MAC. Ringer was add again to flood the cavity.

Sound stimuli and recording. The sound stimuli consisted of pure tone 60 ms long sound pulses shaped in the beginning and at the end by a 8 ms ramp. The pure tone signal was originated in a signal generator (Wavetek FG5000) which was monitored with a counter (FLUKE 1910A). This wave was modulated with the signal from a pulse generator and delivered to a piezo loudspeaker through a power amplifier (B&K type 2706) and a dB-attenuator. These equipments were computer controlled according to a defined experimental program. The stimulus frequencies could range from 2 to 35 kHz and were selected according to the whole nerve threshold curve. Unfortunately many cells proved to be most sensitive below 2 kHz. The sound pressure level at the preparation could be varied in the range 40-110 dB (relative to 20 microPascal) in 5 dB steps. The computer was instructed to scan through a window of frequencies and intensities inside the above ranges. The pulses were repeated 5 times with 200 ms intervals, and a 500 ms interval was allowed before the generation of the next group of 5 pulses with different characteristics. The distance from the loudspeaker to the animal was about 23 cm. The sound pressure of the stimulus was previously calibrated using a 1/4 inch microphone (B&K type 4135) placed close to the preparation and a measuring amplifier (B&K type 2608). The computer equalised the stimuli intensity through a table of attenuation values corresponding to each allowed frequency.

The sound stimulus could be switched to a sample of the calling song. It was played back with a tape recorder and through the same power amplifier, whose built-in dB-attenuator was used to change the song intensities.

The above mentioned microphone and measuring amplifier were used also for sound recording. In this case the microphone was placed at about 2 cm from the preparation, thus reducing the relative importance of the echoes, originated on devices around the animal such as micromanipulators and holders, on the sound produced. The Faraday cage surrounding the set-up was lined with sound absorbing material.

Cell recording device. The cell potentials were amplified (10x) with a microelectrode amplifier (built on the MPIV). The spoon supporting the ganglionic complex was used as an indifferent electrode. The high impedance preamplifier stage was located over the electrode micromanipulator near the preparation, to reduce electrical noise. The cell signals were monitored with oscilloscope and headphones (after fed in an audio amplifier). The microelectrode amplifier allowed current injection either to excite the cell (depolarization) or to stain it by injecting the dye with negative current. The current was monitored with a digital voltmeter. In some cases a small negative current injection (-0.5 to -0.7 nA) was initially used to reduce and stabilize the cell spike activity. Staining the cell was made by injecting a negative current of -1 to -3 nA (occasionally higher currents) from 5 to more than 15 minutes.

Recordings were stored on tape with a RACAL Store 4 DS at a tape speed of either 3 3/4 inch.s-1 or 7 1/2 inch.s-1, responding with FM channels from DC to 2.5 kHz and DC to 5 kHz or with D.R. channels in the ranges 0.1-19 kHz and 0.1-37.5 kHz respectively. Three FM channels received 1) the cell signals, 2) the pulse used for shaping the sound stimulus, and 3) monitored the current injection. The 4th channel was equipped with D.R. electronics for recording the microphone signal. The counter value and the settings of each channel were annotated after each recording session for an easier localization on the tape and analysis.

Preparation of electrodes. The microelectrodes were pulled from thin walled glass pipettes with an inner fibreglass guide. The quality of the tip was first checked with a compound light microscope. The electrodes were then filled with a solution of 3% Lucifer Yellow in 0.25 M lithium chloride. Electrode resistances were usually between 50 Mohm and 150 Mohm.

Cell penetration. A glass electrode mounted on a WPI capsule filled with 0.25 M lithium chloride was positioned, with a LEITZ micromanipulator and with the help of a WILD M5A stereomicroscope, over a selected place on the MAC. The electrode was carefully moved down close to the ganglia, easily detected by headphone monitoring. The microscope, mounted on a

movable arm, was moved away from the preparation and then a sequence of tapping on the micromanipulator and slightly moving it further down started. This procedure was repeated until penetration of an acoustic cell was obtained. An acoustic stimulus was continuously presented in order to immediately recognize most of the auditory interneurons. In other cells a short depolarization was made to check if sound production could be induced by the neuron. From times to times the electrical resistance of the electrode was checked.

Experimental recording protocol. Once an auditory interneuron was penetrated, an experiment was started with the following protocol: a) presentation of acoustic stimuli scanning the frequencies and intensities desired, to obtain the threshold curve characteristics of the cell; b) play-back of a previously defined calling song sample; c) injection of negative current for staining the cell with Lucifer Yellow.

Processing of the ganglia. After staining a cell the MAC was dissected and immersed on Pipe's fixative for 2 hours, washed on Pipe's buffer (2x 10 minutes), and dehydrated in an ethanol series (30%, 50%, 75%, 85%, 90%, 95% ethanol, each step taking 5 minutes, and twice on 100% ethanol during 10 minutes each). The MAC was then transferred to Methyl salycilate for 10 minutes and mounted on a glass slide for observation with a fluorescence microscope. The ganglionic complex was kept on opaque containers to reduce fading of the dye while processing occurred.

A fast drawing was made under microscopic observation for reference and a series of photoslides was taken scanning the deep of the ganglia. Final drawings were made reconstructing the cell through the projection of the photographic series.

The preparation was returned back to 75% ethanol and stored on the fridge.

Data analysis. Counting the spikes induced by the sound stimuli was made adjusting a time window triggered by the pulse signal during which the recording of the cell activity was analysed. The signal was band pass filtered to considerably reduce the amplitude of the excitatory post synaptic potentials and delivered to the input of an adequately adjusted Schmidt trigger connected

to an event counter with digital display. The play-back was slowed down 8 times allowing hand annotation of the counts. The thresholds were judged both with chart prints of the recordings and with the spike numbers increasing (1-2 spikes) above the average of the spontaneous activity. Chart prints were made with a 8 channel chart recorder (PICKER UNISCRIPT UD210) slowed down (8x) during play-back. Other analysis was done with a PC computer and home made software.

Sound Production

1. Foreword

Sound production is a widespread phenomenon in insects and in many cases specialized structures have evolved to generate and broadcast the signals. These are produced in many behavioural contexts (e.g. Alexander 1967; Bailey 1990) but the intraspecific signals allowing reproduction are the most studied so far.

Sound production in the context of intraspecific acoustic communication requires that the signal emitted by the sender is 1) detectable and 2) specific. A comprehensive overview of these problems in the context of an insect acoustic communication system is only available for a few species (for crickets see Huber, Moore and Lower 1989).

1) The requirements for detectability depend as whether the communication is to be established at close or long range. The calling signals of many insects, including cicadas, which are used for the attraction of mates at a distance, need to fulfill certain requirements:

a) The intensity output and therefore the maximum range relative to the costs (e.g. energy costs, predation costs) should be optimised. The efficiency on sound production is related to energy economy which is dependent both on muscular and sound radiation efficiencies. Here the insects face the problem of their own size because sound radiation becomes increasingly inefficient when the wavelength of the sound generated is larger than the size of the animal (Bennet-Clark 1971, 1975; Michelsen 1983). Broadcasting high frequency signals would increase the radiation efficiency in many small insects but, on the other hand, this will reduce the range within which the sound would be received, because of a stronger attenuation over distance (e.g. Lyon 1973). To generate loud sounds insects often concentrate the energy in a narrow frequency band, using resonating systems, as in crickets (e.g. Nocke 1971, Michelsen and Nocke 1974). At least in some cicadas the hollow abdomen opening to the exterior by thin membranes is suspected to work as a resonator as well (e.g. Pringle 1954; Fletcher and Hill 1978; Young 1990; Bennet-Clark and Young 1992). Therefore, insects developed special morphological structures

to generate and broadcast the sound most efficiently and many different mechanisms have been recognized (for an overview see Fonseca 1988; Ewing 1989). They may involve 1) stridulation, 2) timbal mechanisms, 3) percussion, 4) vibration of body structures, or 4) fluid ejection. The frequency range of the sound signals emitted will depend on the mechanism of sound production and on the acoustical characteristics of the structures responsible for sound radiation. Thus, in this context it is important to answer the question: What are the structures involved in sound production, how do they function, and what is the frequency contents of the signal?

Behavioural adaptations may contribute to an increase of the communication range as well. Examples are the acoustical burrows of mole-crickets (e.g. Bennet-Clark 1970), the postures kept by some crickets (Oecanthus) while singing, some of them even cutting adapted holes on leaves (Prozesky-Schulze et. al. 1975), and the elevated singing sites chosen by many orthopterans and cicadas.

However, many other insects avoid the problem of generating loud sounds using different solutions. Certain species are brought together by some other stimulus (e.g. chemical) and the acoustic signals are only used in very short range, as in Drosophila (Bennet-Clark and Ewing 1967, Bennet-Clark 1975) or some Scolitids (Wilkinson et. al. 1967), and to keep a very short sound communication range may be even an advantage in certain cases (e.g. in a bee hive -- Michelsen et. al. 1987). Other insects may induce substrate vibrations for communication with low frequency signals (e.g. small Auchenorrhyncha - Ossiannilsson 1949; Michelsen et. al. 1982; Claridge 1985; Orthoptera - e.g. Busnel et. al. 1955; Kalmring et. al. 1985).

b) The second requirement for a sound signal in intraspecific acoustic communication is that its structure should be maintained on the way to the receiver when distortion is unavoidable. Therefore, acoustic signals to be used in long range communication may have evolved with intrinsic characteristics to cope with distortion. Possibly insects have also developed behavioural mechanisms to reduce distortion, such as the selection of the place from where they broadcast the signal, and the ability for avoiding singing under unfavorable weather conditions (e.g. wind). This problem, however, is not treated in this thesis.

2) An acoustic signal contains intraspecific information which as to be recognized by the receiver. This specificity lies a) in its frequency domain, which depends to a certain extent on the animal's morphology, on the mechanism used for sound generation and on the acoustic characteristics of the structures involved in sound radiation, and b) on its time and amplitude modulation patterns which are mostly dependent on the motor pattern generated by the nervous system. Thus, in order to describe and to understand the evolutionary adaptations of the sender in the acoustic communication it is necessary to evaluate how the interplay of nervous activity and the morphology of the sound production organs determines specificity?

Mechanisms of sound production in cicadas.

Timbal mechanism. The majority of male cicadas use the timbal mechanism to produce sound while the females are usually silent. This mechanism was long ago recognized (see Myers 1929; and Bodson 1976) and described (Pringle 1954; Hagiwara 1955; Moore and Sawyer 1966; Reid 1971; Young 1972, 1973; Popov 1975; Simmons and Young 1978; Young and Josephson 1983a; Weber et. al. 1988). Two ribbed integument membranes -- the timbals, located dorsolaterally in both sides of the first abdominal segment in males, are buckled by two powerful timbal muscles, which are driven by a characteristic motor pattern dependent on the nervous system. These muscles are neurogenic in most of the cicadas studied so far. However, there are also observations suggesting that the timbal muscle may possess myogenic properties (e.g. Pringle 1954). The timbal muscles arise ventrally in an integument invagination -- the chitinous V, and are attached to the timbal through a tendinous ligament. The force developed by the contraction of the timbal muscle overcomes the resistance of the convex timbal, producing an inward deformation which is usually accompanied by sound production (IN pulses). The elasticity of the timbal is responsible for the outward movement, allowed by the relaxation of the muscle, and may produce sound again (OUT pulse). The movement of both timbals may or may not be simultaneous, and, together with the presence of the ribs that may buckle sequentially, up to several IN sound pulses for each rib and often a single OUT pulse may be generated during each muscular contraction cycle. The stiffness of the timbal may be modified by the tensor muscle -- an accessory muscle attached to each timbal frame, and at least in some species it can strongly

modulate the sound produced (Pringle 1954; Hagiwara 1955; Simmons and Young 1978; Hennig et. al. 1994a). In some species the abdominal posture is also responsible for sound modulation. The mechanisms involved in song radiation in cicadas were not much studied but several structures were assigned for sound radiation in different species, such as the timbals (e.g. Michelsen and Nocke 1974), the resonant abdomen (Simmons and Young 1978), and the tympana driven by resonant vibrations set on the internal tracheal air sac (Young 1990; Bennet-Clark and Young 1992).

Sensory information may be collected during singing by sensory chordotonal organs (Young 1975) and it may be used by cicadas as a feedback control to adjust their motor activity. Other sound producing mechanisms found in cicadas. Apart from the timbal mechanism used by the majority of cicadas, several other sound generation mechanisms were recognized so far. Stridulation is present in some cicadas but according to Moore (1973) all known stridulating male cicadas also produce sounds at the same time with the timbals. In the American stridulating cicadas the scrapers (plectrum), situated at the inner bases of the forewings, are rubbed across files (pars stridens) adjacent to them on the sides of the mesonotum. The vibrating wing surface is pointed out as the radiator. In contrast with the timbals, the stridulation structures are present in both sexes and at least in some species the females also stridulate. In addition, wing-wing stridulation mechanisms were found by Boulard (1973) in Ydiella gilloni, a cicada from Ivory Coast. In another system the pars stridens is located in the scutellum of the mesonotum, and the plectrum is in the anal vein of the forewing (Boulard 1976). In 1986 the same author found morphological evidence of supposed stridulating structures in the genitalia of cicadas belonging to the genus Carineta (see also Boulard 1990). Some cicadas make sounds by banging the thickened coastal margins of their forewings against each other. In western North America these cicadas lack timbal organs and females may acoustically respond to singing males (Moore 1973). Other wing-bangers live in South America, Australia and New Zealand, and in these groups the male timbals are also present.

Another mechanism was recognized by Popov (1981) in Cicadetta sinuatipennis, a species where males also have timbals. Here the specialized posterior margin of the forewings lies in a groove of the scutellum of the mesonotum. Part of the wing is bistable and sound is

generated during fast lateral movements of the wings. In *Tibicina quadrisignata* males may also produce sound by rapid lateral movements of the forewings, whose posterior margin also lies in a specialized groove of the mesothorax (personal observations). However, I am not sure if the mechanism involved is the same or if the sound is produced when the forewings clap the hindwings. In this species the sound produced by the wings is certainly much softer and probably of lower frequency than the one generated in C. sinuatipennis. Mechanisms involving the wings were also referred by Myers and Myers (1924), Myers (1929) and by Dugdale and Flemming (1969).

This chapter will describe and experimentally investigate the mechanisms of sound production in six species of timballing cicadas from Portugal comparatively. The specific questions to which this thesis contributes are: 1) How is sound production achieved and what are the adaptations in the sound producing structures of different cicada species? (chapter 2); 2) What are the differences on the song patterns leading to species-specific signals? (chapter 3); 3) What is the motor coordination underlying the sound signals (calling and alarm signals), and does the timbal muscle motor pattern account for all observed acoustic effects? (chapter 4); 4) How is the sound modulation achieved? (chapters 5 and 6); and 5) How is the song radiated and what are the structures involved? (chapter 7). Finally, the conclusion will address the question at what levels in the motor organization of calling song generation in cicadas did evolution act allowing signal diversity and specificity?

2. Morphology of the sound producing structures.

Introduction. The primary sound producing apparatus in cicadas (Fig. 5) is basically formed by the timbals, timbal and tensor muscles (e.g. Myers 1928). The timbals, which may or may not be externally protected by more or less extense timbal covers, are convex integument membranes which are driven by the powerful timbal muscles and are responsible for sound generation. The point of insertion on the timbal of the tendinous structure connected to the timbal muscle is

surrounded by a sclerotized area extending ventrally -- the timbal plate. Furthermore, the timbal membrane is also thickened by the sclerotized ribs, which differ in number and outline among the species. A tensor muscle arising from the metathorax attaches to the anterior region of the timbal frame, sometimes on a differentiated tensor sclerite. The tympana, lying each in an extratympanal cavity, are externally protected by the opercula which project ventrally backwards. They are backed by the internal tracheal air sacs which connect to the outside through the metathoracic spiracles. The air sacs are very large and usually fused in males where they occupy most of the abdomen. The posterior region of the air sac, forming the internal posterior abdominal cavity, is connected to a more anterior one through a passage between the large timbal muscles. This smaller anterior cavity backs the timbals and the folded membranes and is connected to the exterior when the metathoracic spiracles are open. The internal hollow cavities, abdominal wall, tympana, folded membranes, opercula and timbal covering integument folds and possibly also the metathoracic spiracles may contribute to modify and/or radiate the sound generated. Therefore, the study of the morphology and anatomy of the sound producing structures is needed for an understanding of the mechanisms involved in sound production.

The morphology and anatomical features of structures related to the sound production apparatus was observed and compared in *Cicada barbara lusitanica*, *C. orni*, *Tettigetta argentata/atra*, *Tett. josei*, *Tibicina quadrisignata* and *Tympanistalna gastrica*. In *Tettigetta estrellae* only the timbals were observed in detail.

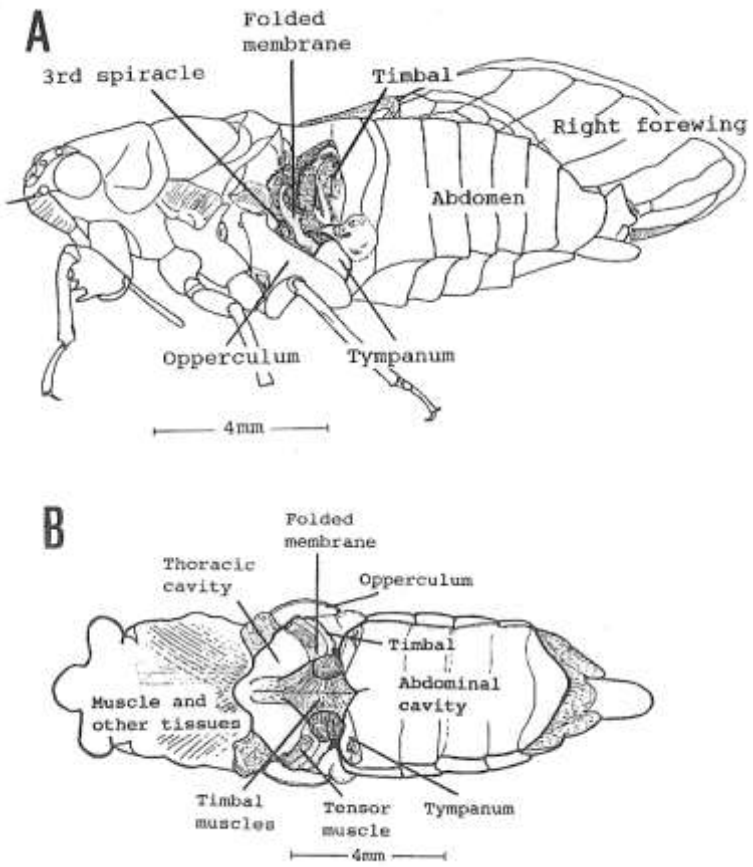


Figure 5 - Sound producing apparatus of a male cicada of the species *Tympanistalna gastrica*. A) Lateral view showing the structures potentially relevant in the radiation of the song. B) Diagram of the internal cavities and sound producing structures as revealed by a longitudinal horizontal section in a view from above.

Results.

Timbals.

C. barbara, *C. orni*: The timbals may be very similar in closely related species belonging to the same genus (e.g. *C. barbara lusitanica* and *C. orni*). These two Cicada species present 3 distinct long ribs. A 4th one, the most anterior, is composed by two halves not connected in the mid dorso-ventral line (Fig. 6 A,B). The first long rib forms a ring. Between the long ribs are 3 small ones situated slightly above the line where the timbal buckles inward. The timbal plate is relatively small in these species. The tensor sclerite is not clearly individualized from the timbal frame and is small compared to the timbal size.

Tib. quadrisignata: Males of *Tib. quadrisignata* show a completely different timbal structure. In this case there are ten long ribs interconnected in the dorsal region of the timbal (Fig. 6 C). This thickened region connecting all ribs ends in a large sclerite juxtaposed to the timbal frame. Between the long ribs are situated nine small ribs along the timbal buckling line. The timbal

plate is relatively small and slender. There is no individualization of the tensor sclerite. In the anterior dorsal region of the timbal frame is situated a structure that probably corresponds to a sensory organ.

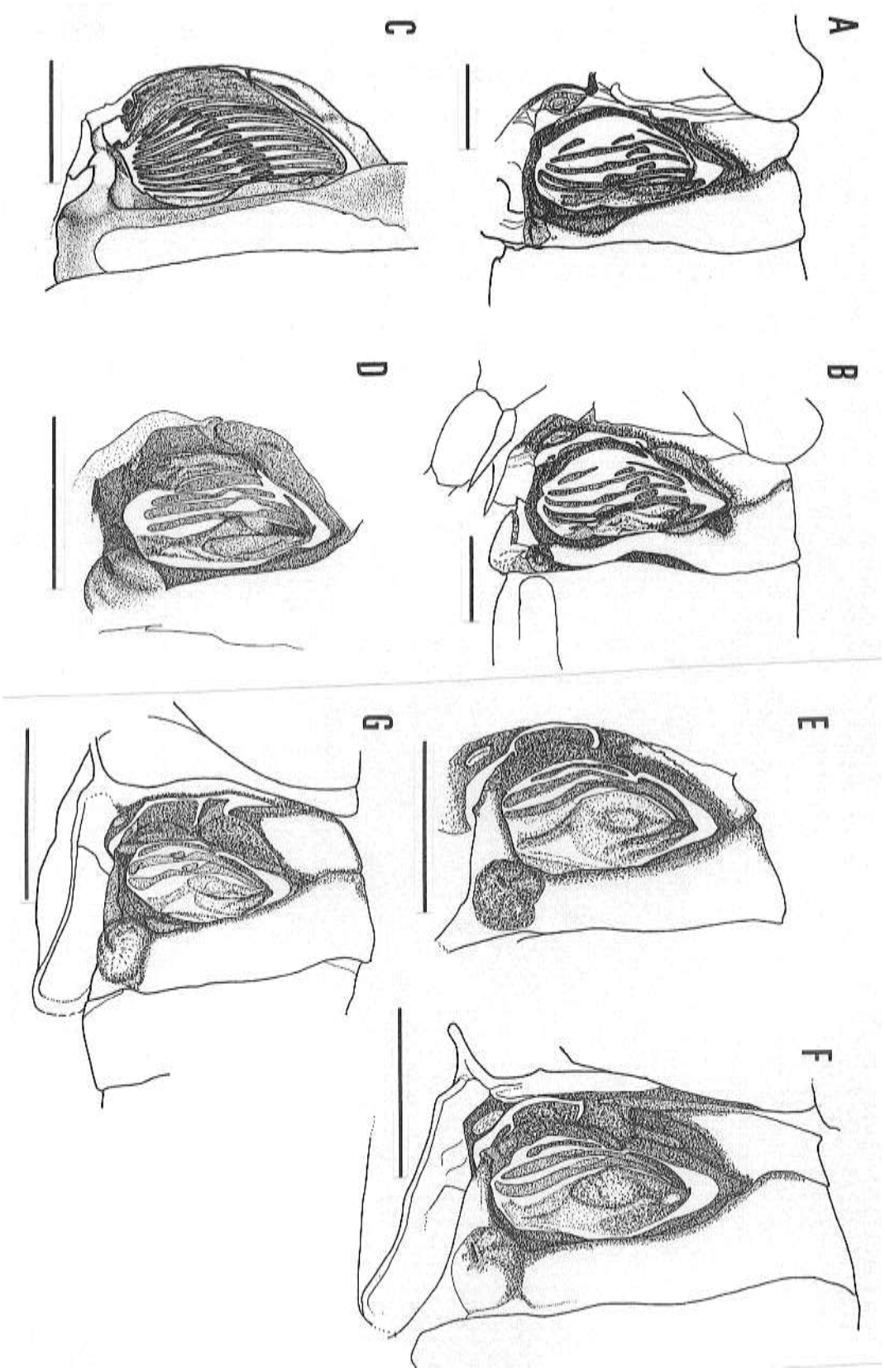


Figure 6 - Morphology of the timbals of the cicada species A) Cicada barbara lusitanica, B) Cicada orni, C) Tibicina quadrisignata, D) Tympanistalna gastrica, E) Tettigetta argentata/atra, F) Tettigetta estrellae, and G) Tettigetta josei, showing the ridded membrane and the timbal frame.

Tettigetta spp.: In contrast to all previous species, in Tettigetta spp. there is a large distinct tensor sclerite surrounded by thinner and flexible folded membrane allowing it relatively large movements (Fig. 6 E-G). The three species observed have two long ribs connected dorsally to each other and a third one anteriorly located and separated from the common sclerotized region at the base of the ribs. Also in the three species there are two small ribs along the line where the timbal buckles, which corresponds also to a region where the long ribs become slender. However, here they are not as separated from the long ribs as in *Cicada* or *Tibicina*. The small ribs are clearly distinct only in *Tett. josei*. In *Tett. argentata/atra* and *Tett. estrellae* they are more or less fused with the 1st and 2nd ribs. The timbal plate is also relatively much larger here than in the previous species.

Tymp. gastrica: Finally, in *Tymp. gastrica*, where the timbal plate is also large, the first three ribs have a general pattern similar to the *Tettigetta*. They are also slender along the buckling line but here there are no small ribs (Fig. 6 D). Anteriorly there is a separated 4th rib, which comes more or less into contact with the timbal frame. The tensor sclerite is also large but different in shape from the Tettigetta ones. As in Tettigetta, movements of this sclerite are allowed by a surrounding membranous region, which is an extension from the folded membrane.

Tensor muscle.

Considering the dimensions of the animals, and of the timbals, the size of the tensor muscle is clearly smaller in *C. barbara lusitanica* and *C. orni* when compared with the other species (Fig. 7 A and B). *Tymp. gastrica* and *Tettigetta* spp. where the tensor sclerite is large and well distinct have correspondingly larger tensor muscles (Fig. 7 D-F). *Tib. quadrisignata* is between the above mentioned species (Fig. 7 C). Since the tensor muscle is attached to the timbal frame, its contraction may influence the mechanics of the timbal, e.g. its convexity and thus the stiffness, and therefore its contraction may change the sound amplitude of the sound pulses generated by the timbals.

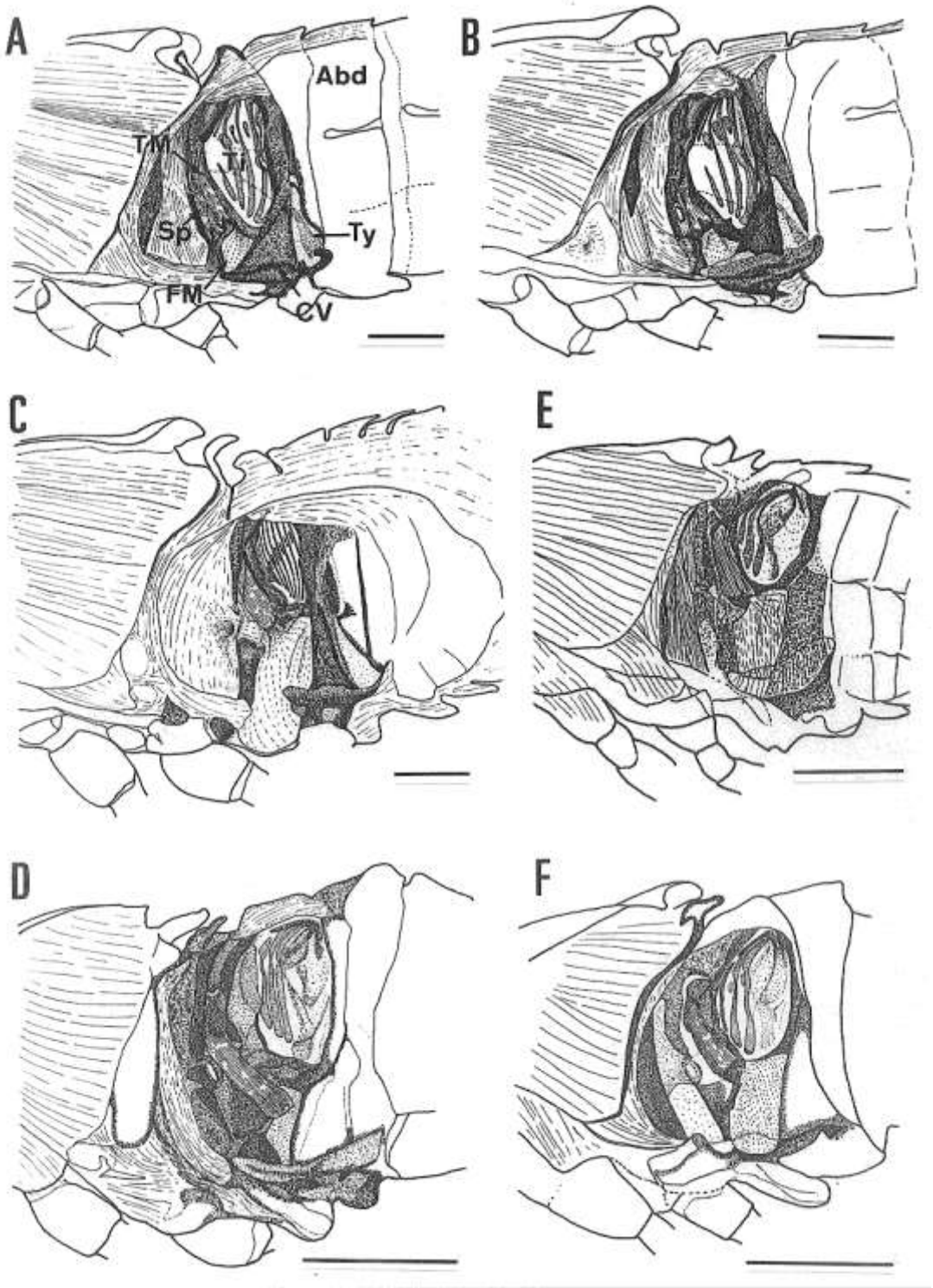


Figure 7 - Diagrams of the timbal apparatus and related structures as revealed by a longitudinal vertical section of male cicadas of the species A) *Cicada barbara lusitanica*, B) *Cicada orni*, C) *Tibicina quadrisignata*, D) *Tympanistalna gastrica*, E) *Tettigetta argentata/atra*, and F) *Tettigetta josei*. The timbal muscles were removed. Ti Timbal, FM Folded membrane, TM Tensor muscle, Sp 3rd spiracle, CV Chitinous V where the timbal muscle arises, Ty tympanum, Abd Abdominal cavity.

Internal cavities, abdomen wall and folded membranes.

The males of all species have a large internal tracheal air sac. However there is a sharp difference in the thickness of the abdomen separating the posterior abdominal air cavity from the exterior. In Cicada spp. and *Tymp. gastrica* the abdomen is very thin except for a narrow dorsal region where the tissues lining is thicker, and for the tip of the abdomen occupied by the reproductive organs. In ageing animals the tissues get dryer and so the wall becomes even thinner. In contrast, in the three *Tettigetta* species and especially in *Tib. quadrisignata* the abdomen wall and the lining with internal tissues are much thicker in all its surface (Fig. 7 C). However, in these thick walled abdomen species the folded membranes are thinner than in the species with a thin abdominal wall. In *Tib. quadrisignata* they are so thin that they become translucent when stretched, which happens naturally in singing animals extending and lifting the abdomen.

Other structures.

C. barbara lusitanica and *C. orni* possess timbal covers, an integument fold which projects anteriorly covering most of the timbal (not shown on Fig. 6). Timbal covers are lacking in the other species. The opercula are very large in males of *Tymp. gastrica* and also in *Tettigetta* spp., being much smaller in *Cicada* spp. and *Tib. quadrisignata* relative to their body size. However, here they can be more tightly adjusted with the edge of the first abdominal segment. In all species the extratympanal cavity may be either widely open or nearly closed by the abdominal movements.

Tympana.

A comparative description of the morphology of the tympana is presented on page 153.

Discussion. The differences observed among the species, as revealed by the comparative descriptions above, suggest: 1) The species with many timbal ribs, as *Tib. quadrisignata*, may be able to generate a larger number of sound pulses in each timbal cycle; 2) In the species

showing relatively large tensor muscles -- *Tettigetta* spp. and *Tymp. gastrica*, which are the same presenting comparatively larger and more independent tensor sclerites, it is likely that the contraction of the tensor muscle may be more important for the modification of the amplitude of the sound pulses than in the remaining species -- *Cicada* spp. and *Tib. quadrisignata*; 3) The abdominal wall may play a different role in sound radiation dependent on its thickness. Similarly, the role of the folded membrane in sound radiation may differ according to its thickness; 5) The male tympana, which are large and backed by the same air sac as the timbals, may play a role in sound radiation as well, as previously suggested by Young (1990). These ideas arising from the morphology of the cicadas will be further studied in the next sections.

In cicada males both sound producing and sound receiving structures are coupled by the one tracheal air sac. This is not the case in the females which do not produce sound. Hence, there is a sex specific difference in the morphology which may have implications for the sound production and radiation by the males. Similarly, it is likely that the sex specific morphological differences may also result in differences of sound perception between males and females (see sound reception).

3. Sound signals

3a. Terminology

To describe an insect acoustic emission it is convenient to be able to specify the behavioural context in which the sound was produced together with a detailed characterization of the physical features shown by the signal.

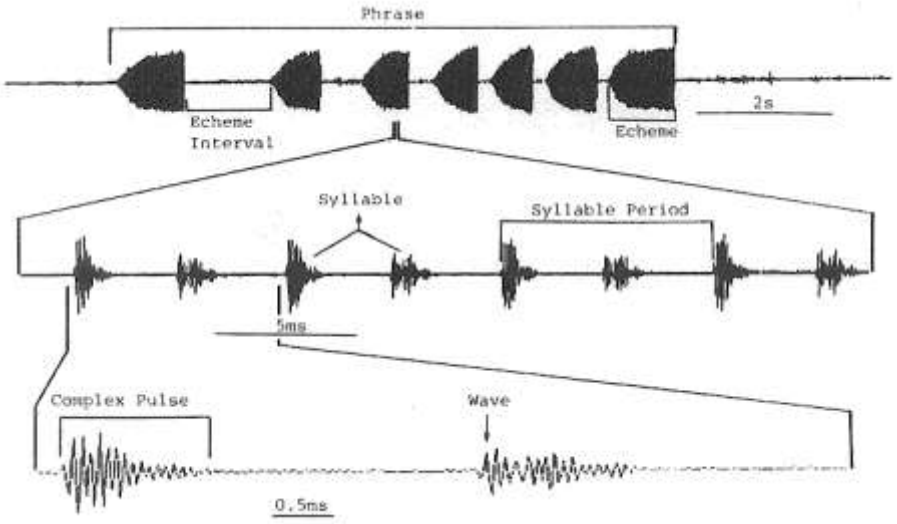


Figure 8 - Oscillograms of the calling song of *Tettigetta estrellae* showing the terminology used in this work.

A strict functional classification is certainly not easy to establish since insects may produce sound in widely diverse behavioural situations. An adaptation of the functional list elaborated by Alexander (1967) is employed throughout this work. For the detailed description of the sound signals generated by the cicadas I use an hierarchic terminology selected by Pinedo (1981) jointly with the terms repertory (e.g. Leroy 1979) and phrase (e.g. Weber et. al. 1988). The sequence and meanings of the terms are illustrated on Figure 8. When requested by an easier understanding of the text, and if not misleading, some other terms were locally adopted.

3b. Sound signals of some Portuguese cicadas

Introduction. A detailed description of the acoustic signals of free behaving animals belonging to a certain species is needed in order to study any aspects linked to their acoustic communication

system. This is especially important, and should be the first step undertaken, when experiments are to be conducted under laboratory conditions since in this case it is essential to be able to recognize deviations from the normal acoustic pattern observed in the field.

In insects the song's characteristics are usually species-specific. Therefore, they shall be carefully described and considered as basic tools for the description of different taxa (Alexander 1967).

The signals are usually produced in certain behavioural contexts, e.g. a male signalling its presence to attract conspecific females at a distance (calling songs), to prepare copulation at close range (courtship songs), or may be produced under distress caused for instance by a predator (alarm signals). The relevant information to the species' communication may be encoded in the time-amplitude pattern and/or frequency domain of the sound signals. The variations of a species' signal are in the time pattern and amplitude modulation characteristics, and sometimes in the frequency modulations as well. Each of these parameters depends on different structures and muscles and on the motor pattern generated by the nervous system. Therefore, the study of the signals may also lead to the formulation of questions related to the functional mechanics of the sound production apparatus as well as to questions concerning the central mechanisms governing cicada sound production.

Here, a description of quantitative and qualitative aspects of the acoustical signals is presented for nine species of cicadas, eight of them among the twelve confirmed earlier as occurring in Portugal (Quartau and Fonseca 1988): *C. barbara lusitanica*, *C. orni*, *Tett. argentata/atra*, *Tett. estrellae*, *Tett. josei*, *Tib. quadrisignata*, *Tymp. gastrica*; and one unidentified species of *Tettigetta*. The results are compared with published data only for C. orni since the acoustic signals of the other species have not yet been described in detail[3]. Moreover, some evolutionary aspects of the relation between the biomechanics of the timbals and the sound produced are discussed.

[3] Fonseca (1991).

Results.

Cicada barbara lusitanica Boulard, 1982. Four different signals were found in this species: a calling song, a courtship song, a male-to-male interaction signal and an alarm signal.

The calling song consists of a continuous train of complex sound pulses (Fig. 9 A), which may last for a long time without interruption. The two timbals (syllables) clearly alternate (Fig. 9 A2), and thus the sound generated by one timbal appears between the pulses corresponding to two consecutive inward movements of the contralateral timbal. The number of sound pulses is reduced when one of the timbals is inactivated (Fig. 9 A2-3, D3-4). During the inward movement of the timbal (IN) three pulses of sound may be usually recognized, and the outward movement (OUT) generates one pulse (Figs. 9 A2-3, D2-4). The damping of those pulses is variable and makes it sometimes difficult to recognize the sound pulses in the calling song. The mode of vibration of the timbal seems to be very complex, since the extended oscillogram shows a waveform with several changes in phase and sudden changes in amplitude (Fig. 9 A4). Frequently the sound produced by the OUT movement of one timbal is superimposed on the IN of the opposite one.

In the beginning of the song, the amplitude rises slowly and reaches the maximum in about 1.5 s at 26.5 ^{o}C. Although both IN and OUT sound pulses become enlarged, the amplitude increase of the IN is more emphasised (Fig. 9 A5). Moreover, the subsequent decrease of the amplitude of the IN seen at the end of a calling song sequence is also more prominent than the variation of the outward sound pulse. The period of the syllables (for one timbal) is temperature dependent. Measurements taken in the middle of song sequences of 7 animals at 26.5 ^{o}C ranged from 8.4 ms (s.d.=0.1, n=100) to 12.4 ms (s.d.=1.2, n=60), which gives syllable rates from 81 s-1 to 119 s-1. Since the timbals are alternating in the song, the overall syllable rates are the double of those values. The average power spectrum (Fig. 10 A) is broad, with most of the energy between 2-3 kHz and 9.5-11 kHz (-20 dB relative to the peak) and a peak at about 5.5-6.5 kHz. The slow amplitude modulation observed in the calling song is accompanied by a frequency modulation (Fig. 9 A1). Extended sonagrams evidence spectral differences between the pulses generated during both timbal cycles. The IN pulses have broader spectra and show lower frequency components not present during the more tuned outward pulse (Fig. 9 A4). The spectral

variations observed during singing are also more prominent in the IN pulses (not shown on the figures).

The courtship song is a continuously amplitude- and frequency-modulated signal produced by a male in the presence of a female or even of another silent male. The sequence illustrated on Figure 9B is repeated over time. In addition one can also see a syllable period modulation. As an example, in one good recording of this song the syllable period changed from 10.6 ms (s.d.=0.2, n=30) in region b to about 20.7 ms (s.d.=1.1, n=30) in region c, with the peak frequency slightly changing from 6.2 to 5.8 kHz. The IN/OUT amplitude relationships are generally similar to the variations observed in the calling song (Fig. 9 B2-3), possibly with stronger variations of the IN pulses during the courtship. Modifications of the power spectra from the soft to the loud regions of the courtship are quite dramatic (Fig. 10 C)

This species has a male-to-male interaction signal (Fig. 9 C), which is usually produced by a male when a singing male starts to court him, and sometimes also when another male starts the calling song very nearby (probably related to the high intensity of the signal). It is characterized by short (about 200 ms) and irregular sound sequences (Fig. 9 C2) produced with irregular intervals during the courtship or even longer. The amplitude of the sound pulses is also variable. The frequency spectra is in the range of the signal of the courting male (see sonagram on Fig. 9 C1 and Fig. 10 D). It is unknown if the courting male is able to discriminate the interaction signal during its own singing. However, my observations of behaving males suggests this possibility, since ceasing of continuous singing activity by the courting male appears to be induced by the interaction signal of the courted male.

The alarm signal, produced by a disturbed animal when seized, may be very irregular in time (Fig. 9 D), in the amplitude of the sound pulses (Fig. 9 D2-3) and in frequency pattern (sonagram on Fig. 9 D1). The syllable period for one timbal in three animals was 12.6 ms (s.d.=0.2, n=100), 12.6 ms (s.d.=0.2, n=100) and 15.2 ms (s.d.=0.3, n=100).

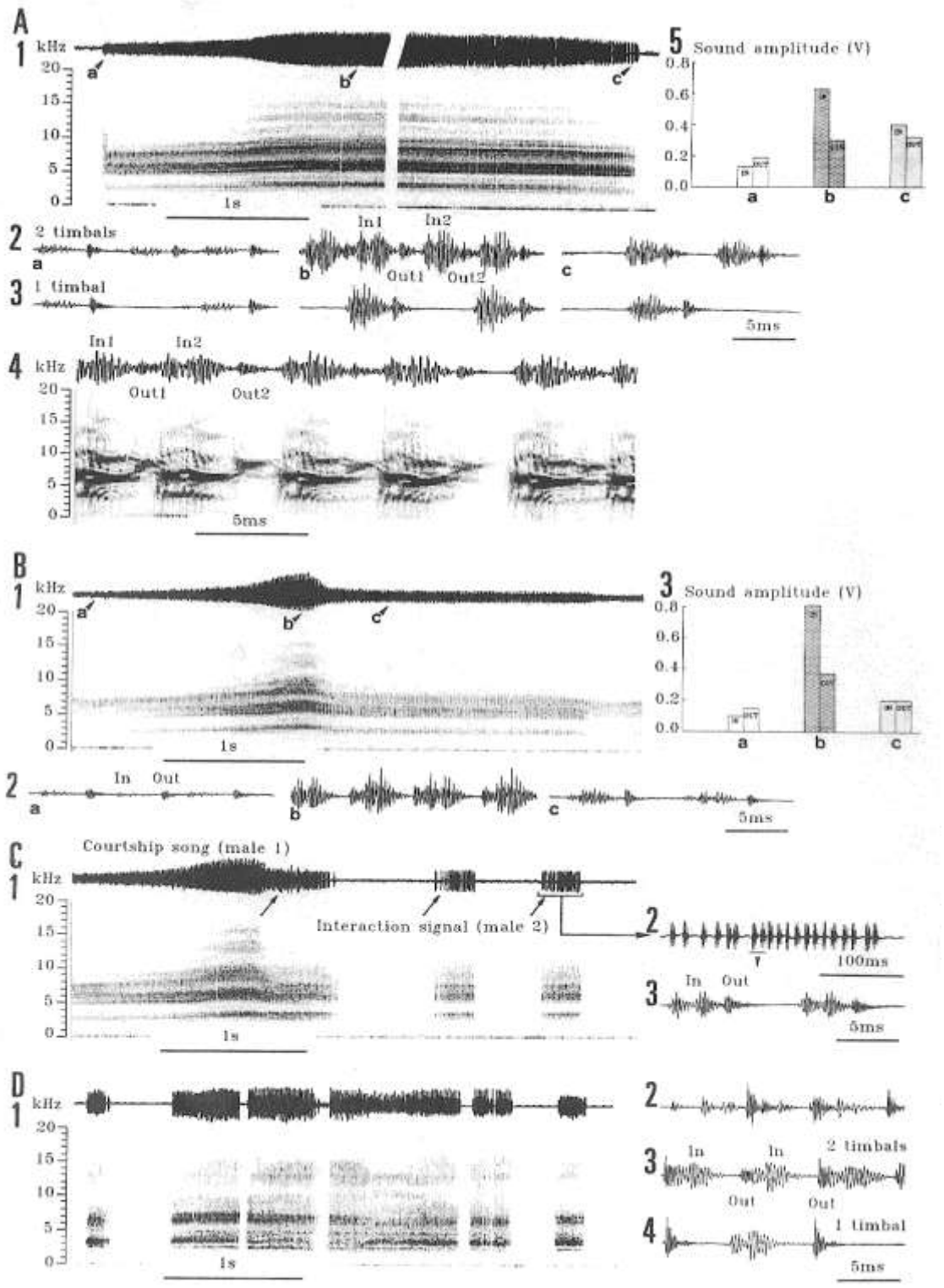


Figure 9 - Sound signals of *Cicada barbara lusitanica*. A) Calling song. 1 - Oscillogram and sonagram with the amplitude and frequency modulations seen on a sound sequence. 2,3 - Extended oscillograms with both timbals working or after the removal of one timbal, respectively, showing in detail a) the beginning, b) the middle , and c) the end of the sound sequence. 4 - Extended oscillogram and sonagram at the middle of the sound sequence revealing the differences in the frequency contents between the IN and OUT pulses. 5 - Variations of the IN and OUT sound amplitude along the calling sequence. B) Courtship song. 1 - Amplitude and frequency characteristic variations of an extract of the courtship song, which is repeated over time. 2,3 - Extended oscillograms and histogram showing the amplitude variations of the IN and OUT sound pulses a) at the beginning, b) in the middle, and c) at the end of the sequence. C) Interaction signal. 1 - Sound of a male (indicated by the arrows) when courted by another male cicada. The frequency contents is included in the courtship song sonagram. 2,3 - Detail of the waveform as revealed by extended oscillograms. D) Alarm signal. 1 - Sequence exhibiting irregular time and frequency patterns. 2,3 - Details of the waveform showing irregular IN and OUT amplitudes. 4 - One timbal signal in the same animal.

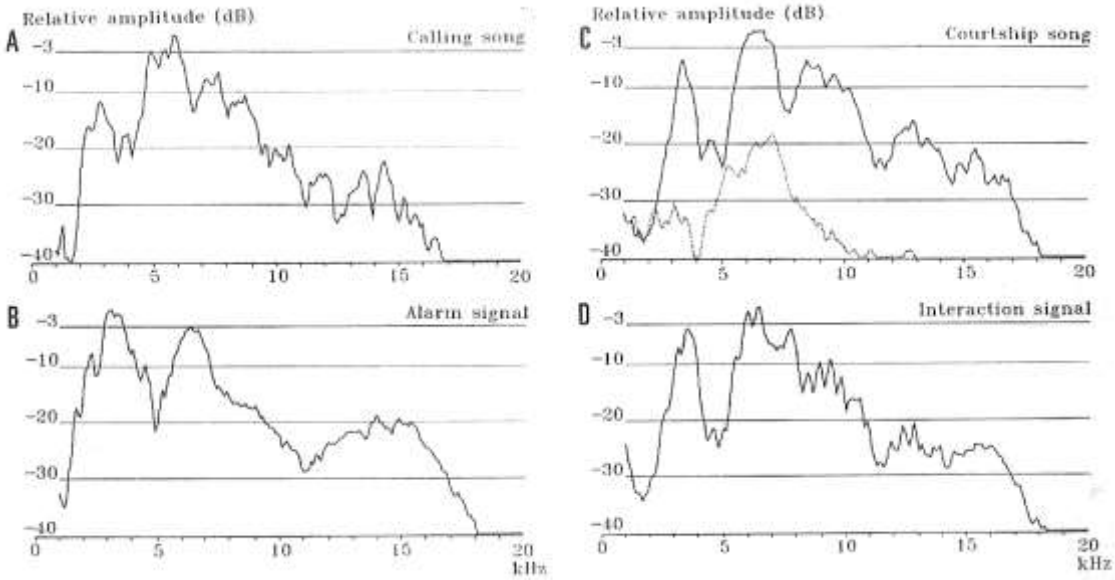


Figure 10 - Averaged log power spectra of the sound signals generated by one male of *Cicada barbara lusitanica*. A) Calling song. B) Alarm signal. C) Spectra averaged in the softer (dotted line) and in the louder (solid line) segments of a courtship sequence. D) Interaction signal.

Compared with the calling song of the same animals, the period is statistically longer ($p<0.01$). The average power spectrum of the alarm song with a peak power usually between 3 kHz and 3.5 kHz (Fig. 10 B) reveals relatively more energy in the lower frequency range than the spectrum of the calling song for the same animals.

Cicada orni Linnaeus, 1758. The calling song consists of trains of sound pulses (Fig. 11 A) delivered periodically (echemes). The relation between the oscillograms and the actions of the timbals is similar to that found in *C. barbara lusitanica*. The inward movement of the timbal may produce three pulses of sound, which are difficult to recognize in the calling song (Fig. 11 A3) but are readily seen in the alarm signal (Fig. 11 B2-3), followed by one pulse related to the outward movement. In the calling song the pulses are more or less fused in a complex waveform presenting several changes in phase and sudden changes in amplitude (Fig. 11 A3). Usually the softer pulse produced in the OUT of one timbal is superimposed on the louder IN of the opposite one, except in the very beginning of each echeme, with the timbals alternating (Fig. 11 A3). The alternation of the timbals is demonstrated by inactivating of one timbal during the alarm signal (Fig. 11 B2-3).

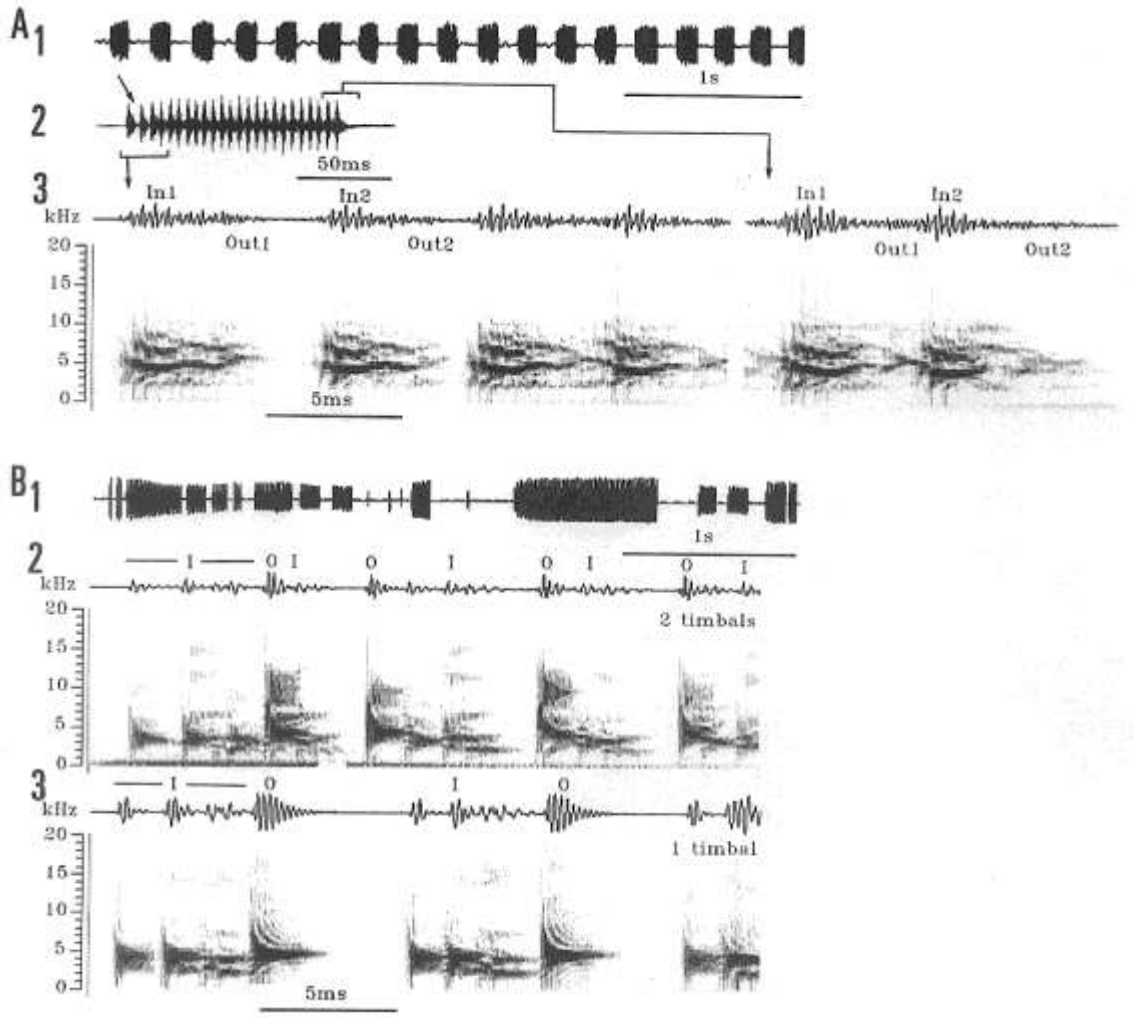


Figure 11 - Sound signals of *Cicada orni*. A) Calling song. 1 - Oscillogram of a sound sequence. 2 - Detail of an echeme showing the characteristic longer interval separating the first syllable from the others (arrow). 3 - Extended oscillograms and sonagrams of the beginning and the end of the echeme. The IN and OUT pulses are easily recognized by their frequency contents. B) Alarm signal. 1 - Sequence exhibiting its irregular time pattern. 2,3 - Details of the waveform and respective sonagrams before and after the removal of one timbal.

The calling song of the five males, recorded at 29-31 ºC, had a quite regular period for the overall succession of timbal actions. Values ranged from 4.1 ms (s.d.=0.1, n=30) to 4.8 ms (s.d.=0.1, n=30), giving syllable periods between 8 ms and 10 ms. The first syllable of each echeme is usually separated by a small interval (see the arrow in Fig. 11 A2). The number of syllables per echeme is variable (extreme values were 10-30), even within the same song. The duration of and interval between the echemes were variable (duration: 45 ms-128 ms, interval: 43 ms-104 ms). It is interesting that four animals recorded at the same time showed similar echeme periods (averages from 148 ms to 155 ms, and standard deviation less then 5% of the average value), but with very different echeme durations and intervals. The average power spectrum is relatively broad (Figs. 11 A3, 12 A), with a peak frequency around 4.5 kHz. The

frequency band, measured at -20 dB relative to peak level, ranged from about 2 kHz to 8.5-10.5 kHz. The IN and OUT pulses may have different frequency contents.

An alarm signal (Fig. 11 B), very irregular in time and sometimes also in amplitude patterns, is produced by a male when seized. The relative amplitudes of the IN and OUT pulses may also change during the generation of the signal. The syllable period of the alarm signal was longer ($p<0.01$) than that of the calling song in two animals for which it was possible to record the two sounds in sequence. The power spectrum (Fig. 11 B) in three animals shows more energy at lower frequencies than the calling song, with the peak between 3 kHz and 4 kHz, and a frequency band extending from 1.5-2 kHz to 6.5-13 kHz. These spectral differences are well demonstrated by the expanded sonagrams (compare Figs. 11 A3 and 11 B2-3). The spectral components of the inward sound pulses produced during the alarm signal are restricted to lower frequencies than shown by the calling song.

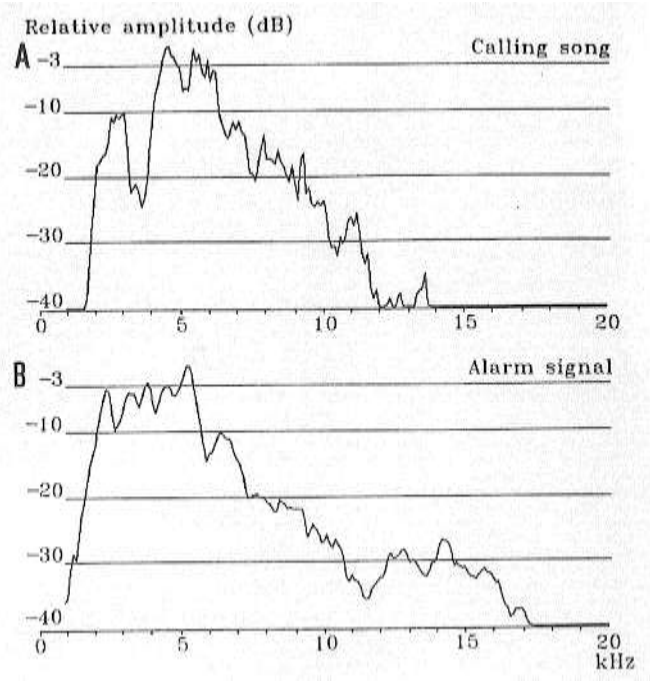


Figure 12 - Averaged log power spectra of the A) calling song and the B) alarm signal generated by one male of *Cicada orni*.

Tettigetta argentata (Olivier, 1790) / *atra* (Gomez-menor, 1957). The calling song (Fig. 13 A) consists of echemes delivered periodically, and may last for a long time. Each echeme is the result of three timbal cycles and the sound produced has a regular amplitude modulation pattern

(Fig. 13 A2-3). The actions of the two timbals are usually slightly separated in time, but the IN sounds of the last syllable are often synchronized (sometimes the last two syllables), as demonstrated by removing one timbal (Fig. 13 A4). Each timbal produces one IN and one OUT pulse. The amplitude of the IN pulse is modulated as it becomes progressively higher toward the end of the echeme. The damping of the pulses is variable but always high. Measurements of the calling song of fifteen animals at temperatures from 20 ºC to 33 ºC showed a strong dependence of the syllable period on the temperature. The echeme period, also temperature dependent, is quite constant in each animal at a certain temperature, but sometimes the animal produces one or two echemes with much longer periods (see Fig. 13 A1).

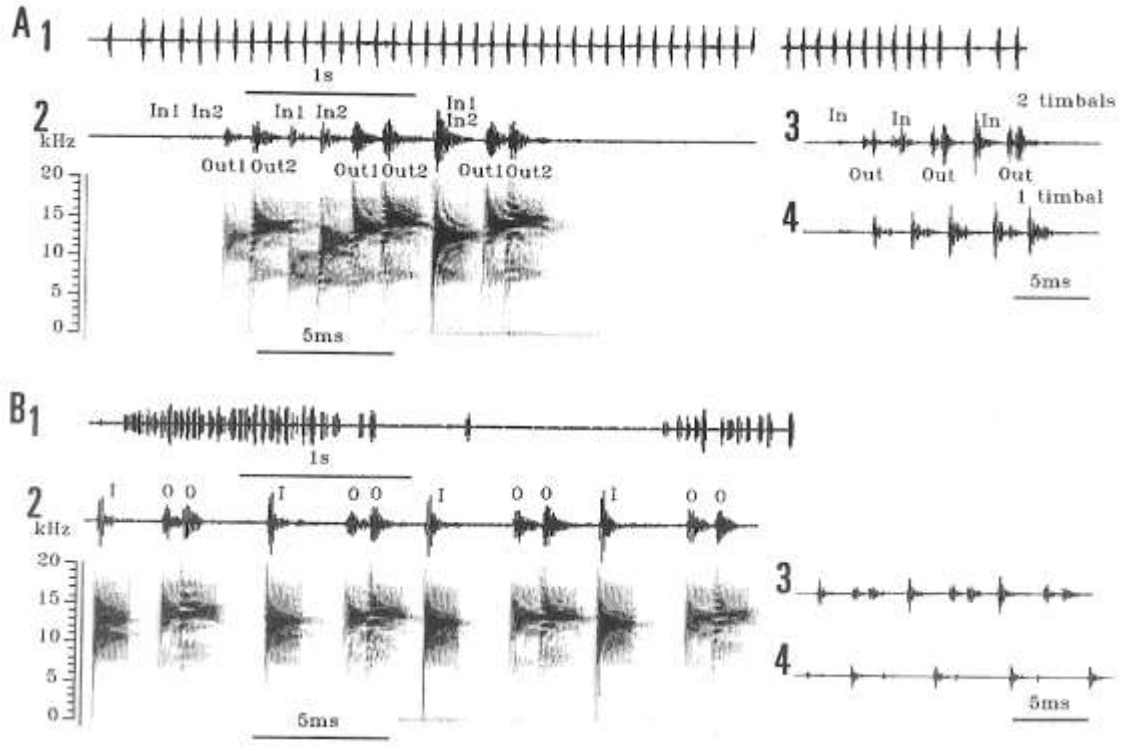


Figure 13 - Sound signals of *Tettigetta argentata/atra*. A) Calling song. 1 - Oscillogram of a sound sequence. 2 - Extended oscillogram and sonagram of an echeme revealing the differences in the frequency contents between the IN and OUT pulses. 3,4 - Extended oscillograms of a characteristic echeme formed by three syllables before and after removing one timbal, respectively. B) Alarm signal. 1 - Sequence exhibiting irregular time and frequency patterns. 2 - Details on the amplitude and frequency contents of the IN and OUT sound pulses as revealed by an extended oscillogram and respective sonagram. 3,4 - Echemes exhibiting different number of syllables and amplitude patterns. In 4 there is only one timbal working.

The number of echemes between these unusually long periods is variable (I counted from 16 to more than 100). The extreme values of the echeme period were 90 ms (s.d.=7.7, n=100, at 21 ºC) and 61 ms (s.d.=2.5, n=100, at 25 ºC) (excluding the unusually long periods). Unexpectedly, the smallest value obtained in the calling song of an animal was at only 25 ºC. However this was

observed in an animal collected in a windy zone where the temperature usually does not reach high values. The syllable period measured between OUT pulses ranged from 7.6 ms (s.d.=0.4, n=100, at 20 ºC) to 4.2 ms (s.d.=0.1, n=100, at 29 ºC). The average power spectrum (Fig. 14 A) is broad, with maximum energy at 13-14.5 kHz, and a frequency band from about 5.5-16.5 kHz (-20 dB relative to peak). An extended sonagram of an echeme illustrates the spectral differences between IN and OUT sound pulses (Fig. 13 A2). It seems to occur an increase of the frequency of the IN pulses toward the end of the echeme while the frequency contents of the OUT pulses remain more stable.

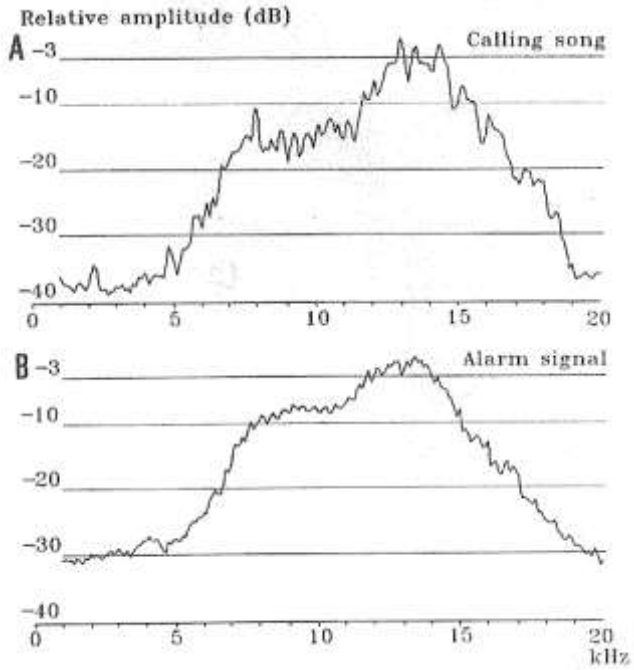


Figure 14 - Averaged log power spectra of the A) calling song and B) alarm signal generated by one male of *Tettigetta argentata/atra*.

The alarm signals have a highly irregular time pattern (Fig. 13 B1). When the animals are disturbed they may produce these sounds as they fall from the supporting plant down into the vegetation, where they remain quiet. The echemes include often 4 syllables and the amplitude relationships between IN and OUT pulses is variable (cf. Fig. 13 B3-4). However, it is usually not seen a steadily increase of the amplitude (and frequency) throughout the echeme (Fig. 13 B2-4), and the IN amplitude is often large from the beginning. The power spectrum may shift slightly

toward lower frequencies but in many cases remains essentially similar to the calling song one (Fig. 14 B).

Tettigetta estrellae Boulard, 1982. The calling song of this species consists of a repetition of complex phrases. At the very beginning of sound emission each phrase is composed of two or three sequences of syllables (echemes) soon extending to the usual 5-7 echemes of different duration (Fig. 15 A1). In the more frequent pattern the phrase starts with an echeme of 1.0-1.7 s in duration separated from the following 4-6 by a relatively long interval (1.3-2.5 s). The following echemes are usually shorter except the last one (sometimes the last two) which can be as long as the first echeme (0.9-1.8 s). The intervals also get progressively shorter from the beginning to the end of the phrase. The actions of the two timbals are synchronized or slightly separated in time (Fig. 15 A2-3). Each timbal produces one pulse during the inward and another one during the outward movement (Fig. 15 A2-3). In the beginning of each echeme the amplitude rises continuously to reach the maximum after 0.2-0.6 s. Both IN and OUT pulses strongly increase at the beginning of the echeme.

The calling songs of five animals recorded at temperatures between 28 ^{o}C and 30 ^{o}C showed a quite regular syllable period of 7.4 ms (s.d.=0.1, n=100) to 9.1 ms (s.d.=0.3, n=100). The period of the phrase ranged between 11 s and 13.5 s and the phrase interval was about 2.5-3.5 s. The average power spectrum (Fig. 16 A) is broad with maximum energy around 11.5-14 kHz and a frequency band extending from 5.5 to 16 kHz (-20 dB related to peak). At the beginning of each echeme there is clear frequency modulation (Fig. 15 A1). Throughout the echeme the energy increases considerably toward the higher spectral components and the song becomes more tuned (Fig. 16 A). The spectral composition of the IN and OUT pulses appears only slightly different in an extended sonagram (Fig. 15 A2).

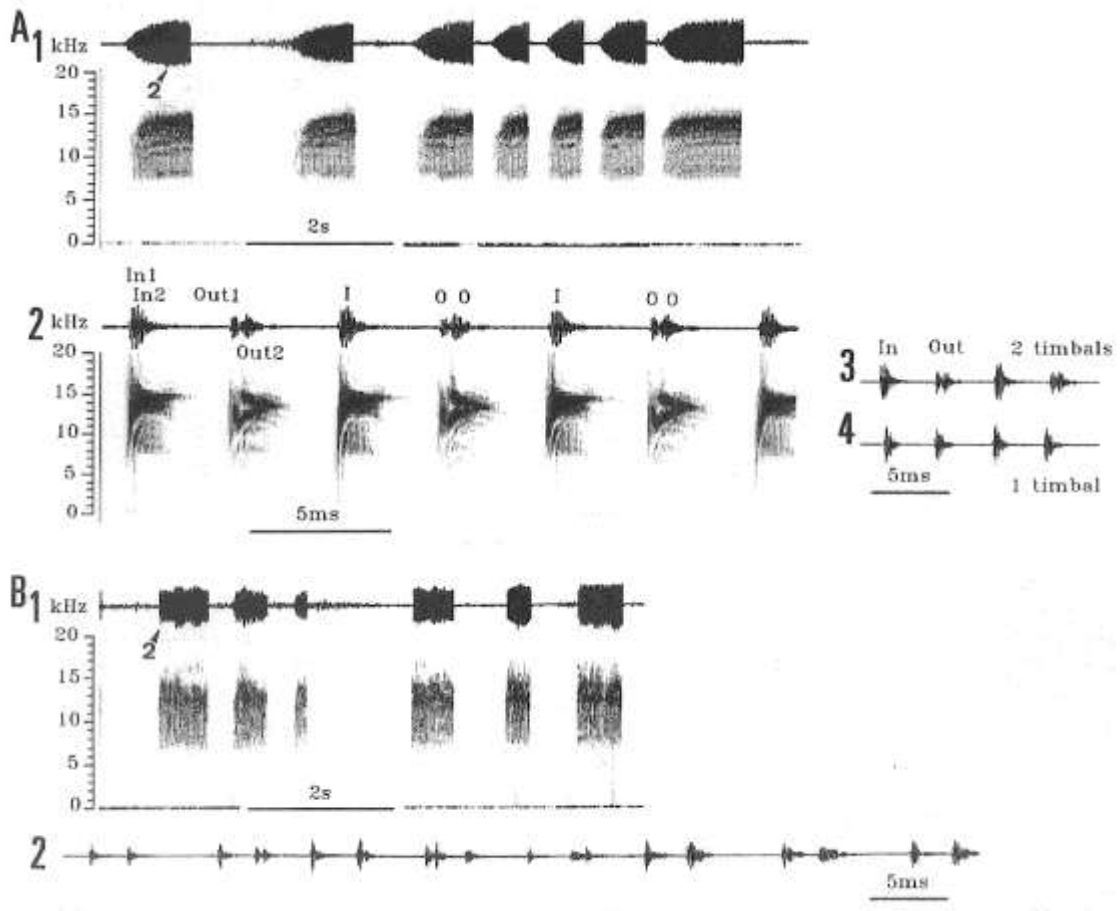


Figure 15 - Sound signals of *Tettigetta estrellae*. A) Calling song. 1 - Oscillogram and sonagram of a phrase with the characteristic amplitude and frequency modulations. 2 - Extended oscillogram and sonagram revealing the frequency contents of the IN and the OUT pulses in the loud region of an echeme. 3,4 - Extended oscillograms of two timbal cycles before and after removing one timbal, respectively. B) Alarm signal. 1 - Oscillogram and sonagram of a sequence exhibiting irregular time pattern. 2 - Extended oscillogram showing irregular time pattern and amplitude of the pulses.

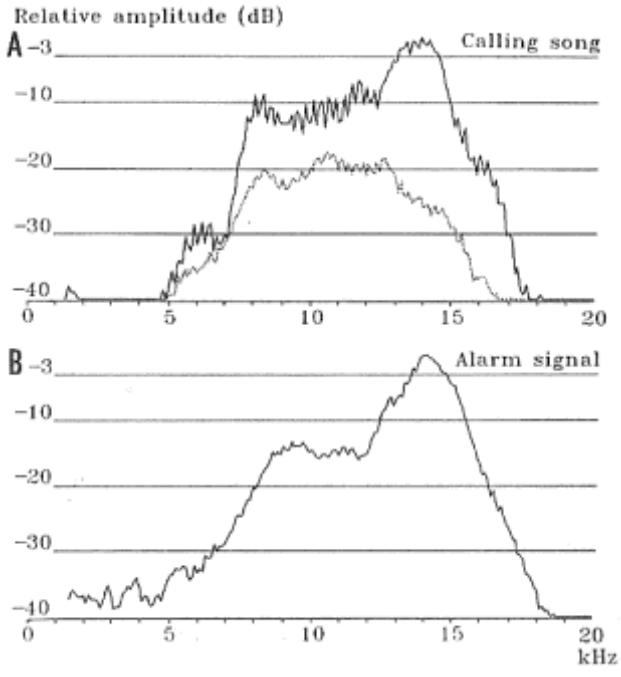


Figure 16 - Averaged log power spectra of the A) calling song measured in the soft beginning of an echeme (dotted line) and in the louder segment (solid line), and of the B) alarm signal generated by one male of *Tettigetta estrellae*.

The alarm signal lacks the characteristic amplitude and frequency increase in the beginning, and has an irregular time and amplitude pattern (Fig. 15 B). Pulse damping seems to be higher than in the calling song and sometimes the timbal actions are not synchronized (Fig. 15 B2). The averaged power spectrum (Fig. 16 B) is essentially similar to the calling song one.

Tettigetta josei Boulard, 1982. I have identified three signals in this species: the calling song, the courtship song (not recorded) and an alarm signal.

The calling song (Fig. 17 A) consists of the repetition of long sequences of syllables -- phrases, separated by short intervals. Each phrase is usually composed of two distinct parts. Part I is a long sequence of syllables delivered in groups of two -- echemes (Fig. 17 A2-4 a,b). In the beginning of the phrase the amplitude of the pulses rises slowly to a fairly constant maximum. Part II, at the end of the phrase, is short. Syllables are produced continuously (Fig. 17 A2-4 c) and the syllable interval is reduced to the end of the phrase. The action of the two timbals is synchronized or little separated in time, and each timbal can produce one pulse during the IN and another one in the outward movement. The IN pulse can vary greatly in amplitude; it strongly increases from the first to the second syllable within each echeme on Part I (Fig 17 A1b) and is always loud on Part II (Fig. 17 A2-4 c). The relatively soft OUT pulses remain stable throughout the phrase. The damping of the pulses is very high.

Calling songs of seven animals recorded at 24-27 ^{o}C show very constant echeme periods in Part I for each animal, with average values from 22 ms (s.d.=1.2, n=100, at 27 ^{o}C) to 25.6 ms (s.d.=1.1, n=100, at 26.5 ^{o}C). At the end of Part I this period becomes smaller. The syllable periods were difficult to measure from the songs recorded in the field since the first IN pulses of each echeme were frequently so soft that they didn't show on the oscillograms. In Part II, where the echeme period corresponds to the syllable one, the period ranges from 7.1 ms (s.d.=0.9, n=36, at 24 ^{o}C) to 10 ms (s.d.=1.2, n=11, at 26.5 ^{o}C).

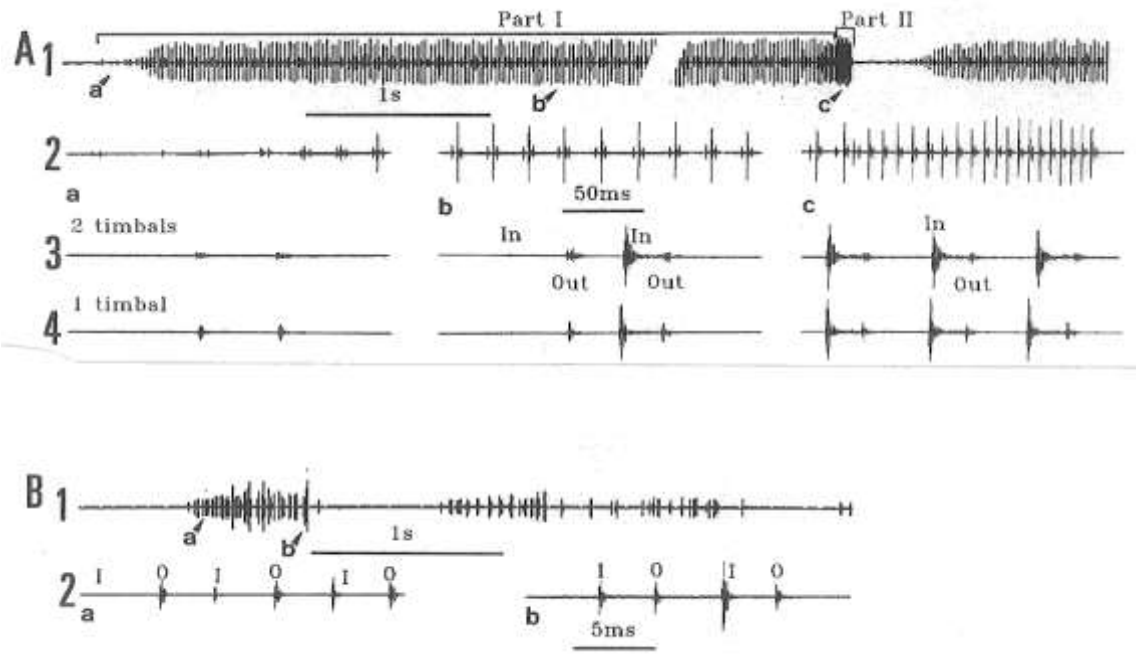


Figure 17 - Sound signals of *Tettigetta josei*. A) Calling song. 1 - Oscillogram of a phrase. 2 - Detail of the echemes a) on the beginning and b) at the middle of Part I, and c) on Part II of the phrase. 3,4 - Extended oscillograms of songs obtained before and after removing one timbal, respectively, revealing the characteristic variations on the amplitude of the echeme sound pulses a) at the beginning and b) on the middle of Part I, and c) on Part II of a phrase. B) Alarm signal. 1 - Sequence exhibiting irregular time pattern. 2 - Details on the number of syllables and amplitude variations of the pulses within two echemes (a and b).

The large standard deviation is due to the progressively shortening of the periods in this part of the phrase. Some experiments subjecting the same animal to different temperatures showed a clear dependence of the syllable and echeme periods on temperature. The duration of the phrase is highly variable both in the same animal and in different animals, and I measured times from 2.6 s to more than 24 s. The interval between phrases had values from 0.2 s to 0.5 s, and the duration of Part II, much more constant, gave values from 125 ms to 195 ms among the signals studied. At the beginning of the phrase, maximum amplitude is reached in 0.2-0.6 s. The broad power spectrum of the calling song was at its peak at 15-16 kHz, which was the upper limit of the linearity of my audio equipment. Using a bat detector in a tuned mode I found that the spectrum reaches the ultrasonic range, but this part of the spectrum was not studied.

Animals observed in courtship behaviour in the field had a song with shorter phrases delivered at a higher cadence. Following physical contact singing activity was terminated and copulation started immediately.

Alarm signals emitted when the animal is seized have a highly irregular time pattern (Fig. 17 B1). The echemes have often three syllables and the amplitude modulation pattern within the

echemes is irregular (Fig. 17 B2). The spectrum is broad and the pitch seemed to be lower than that of the calling song.

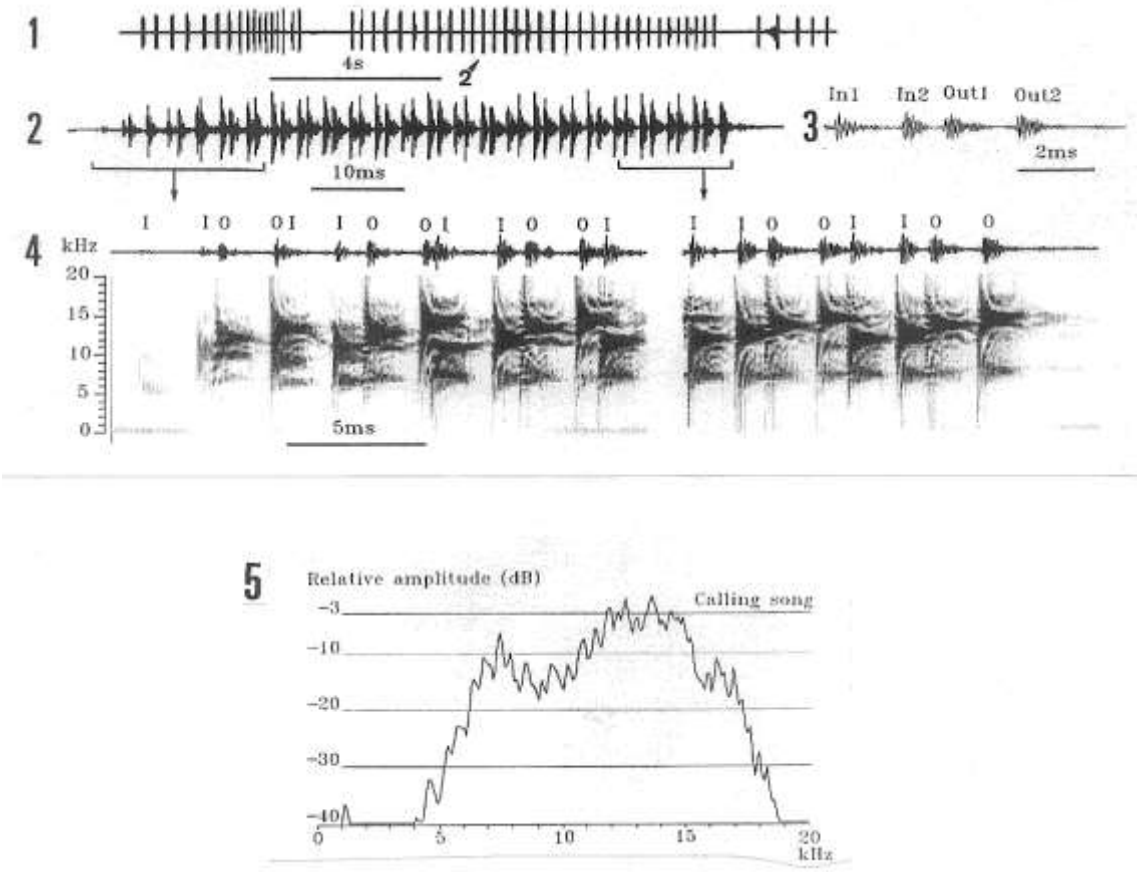


Figure 18 - Calling song of Tettigetta sp. 1 - Oscillogram of a sound sequence. 2 - Amplitude modulation within an echeme. 3 - Detail of the waveform of the sound pulses generated by both timbals. 4 - extended oscillograms and sonagrams of both the beginning and the end of an echeme, revealing the variations in amplitude of the sound pulses at the beginning of the echeme and differences in the frequency contents between the IN and OUT pulses. 5 - Averaged log power spectrum of the calling song.

Tettigetta sp. I have found this species only in the southeast of Portugal, in the region of the Castro Marim marshes. I have not yet been able to identify it. Only one animal was recorded, but I think that it is interesting to include it because the calling song is clearly different from the songs of the other species.

The calling song consists of a sequence of echemes (Fig. 18-1), the duration and interval of which are variable, becoming shorter at the end. The duration of the echeme was 40 ms to 100 ms at 26 ^{o}C, but most frequently near 80 ms. The period, around 0.5 s in the beginning, decreased to 0.2 s or even further at the end of these sequences. The sequential order of timbal actions was

IN-1, IN-2, OUT-1, OUT-2 (Fig. 18-3,4). The corresponding sound pulses were spaced with a period of about 1.5 ms. The syllable period was near 6 ms. The average power spectrum (Fig. 18-5) was broad with maximum energy around 13.5-14 kHz and a frequency range from near 6-16.5 khz (-20 dB related to peak). The IN and OUT pulses could be discriminated in an extended sonagram (Fig. 18-4), since the OUT pulses exhibited higher main frequencies.

Tibicina quadrisignata (Hagen, 1855). Calling song: The sound is mainly produced during the inward movement of the timbal, with each pulse being clearly related to the buckling of one long rib. The outward movement produces a very weak sound (Fig. 19 A3). The deformation of the timbal follows a line defined by a series of small ribs placed between the long ones. Both timbals alternate, as shown by inactivating one of the timbals (Fig. 19 A2-3). Usually the last IN pulses and the weak OUT pulse produced by one timbal are superimposed on the first pulses of the opposite one.

The calling song (Fig. 19 A) is composed of long sequences of sound pulses which may last for several minutes. In the beginning of each sequence the amplitude rises slowly and stabilizes after about 0.5-0.8 s. This correlates with the observed behaviour of the animal, which elevates the abdomen, lifts the wings, and keeps this position during sound emission. The number of pulses per timbal action is also enhanced (Fig. 19 A2-3), indicating an increase in the number of buckling ribs, which is possibly related to an increase in the amplitude of the movement of the timbal, or an increase in its stiffness. Measurements of the calling song of five animals at temperatures between 29 ^{o}C and 32 ^{o}C show that syllable periods are very constant for each animal, ranging from 17.5 ms (s.d.=0.3, n=60) to 19 ms (s.d.=0.2, n=100). The pulse period changes in a systematic way during a syllable. Averages were between 1.3 ms (s.d.=0.1, n=100) and 1.6 ms (s.d.=0.1, n=100). The average power spectrum (Fig.20 A) is broad, although more tuned than in the other species described here, and with a maximum usually at 8.5-10.0 kHz, and a -20 dB range (related to peak) from

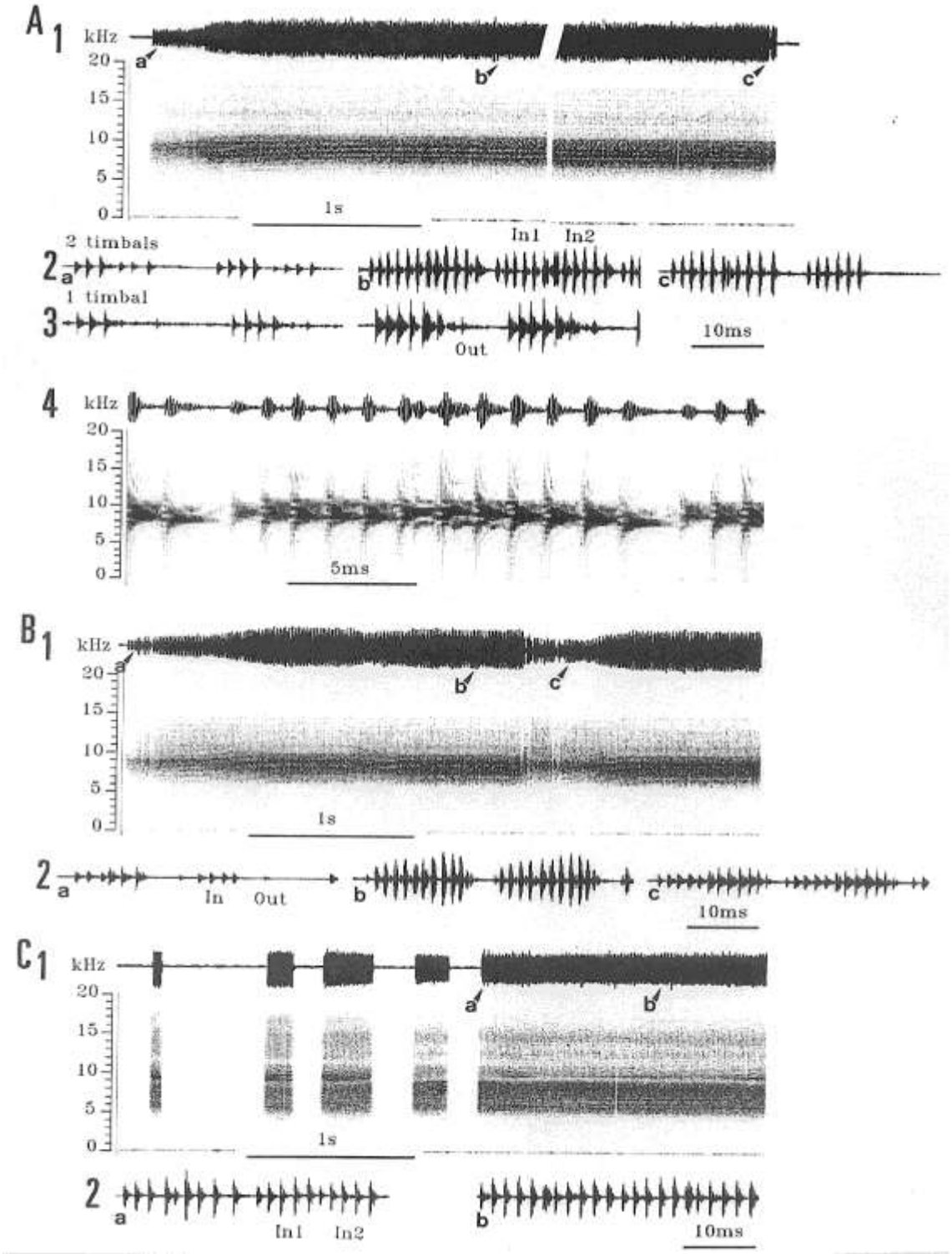


Figure 19 - Sound signals of *Tibicina quadrisignata*. A) Calling song. 1 - Oscillogram and sonagram with the amplitude modulation and relative constancy of the frequency seen on a sound sequence. 2,3 - Extended oscillograms with both timbals working or after the removal of one timbal, respectively, showing in detail a) the beginning, b) the middle , and c) the end of the sound sequence. 4 - Extended oscillogram and sonagram of the pulses generated during the inward buckling of both timbals. B) Courtship song. 1 - Amplitude variations and frequency contents of an extract of the courtship song. 2 - Detail of the sound pulses in three segments of the song with different loudness (a, b, c). C) Alarm signal. 1 - Sequence exhibiting irregular time pattern and its frequency contents. 2 - Details of the syllables a) in the beginning and b) at the middle of a sound sequence.

6.5-12.0 kHz. The sonagrams show a stability of the sound frequency both within a syllable (Fig. 19 A4) and during all the song sequence (Fig. 19 A1).

Figure 19B shows a short sequence of the courtship song. The calling male starts his courtship as soon as a female flies nearby. This song is

composed of a series of amplitude modulated sequences correlated with movements of the abdomen as has been reported for other species by e.g. Young (1972) or Weber et. al. (1988). At the beginning of each sequence the animal makes an audible sound with the wings. Usually the courtship song lasts for the short time needed for the animals to get together. Then the male stops singing and copulation starts. In this case the amplitude modulation of the sound is not accompanied by a modulation in frequency (Fig. 19 B1).

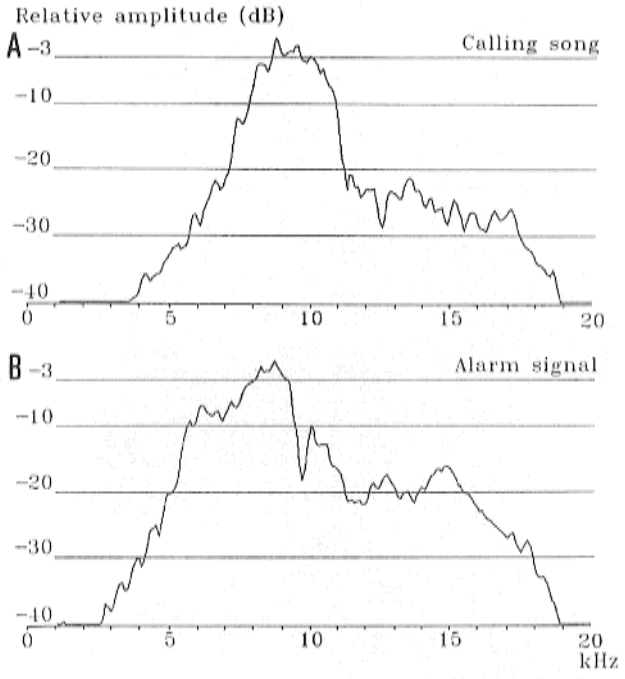


Figure 20 - Averaged log power spectra of the A) calling song and the B) alarm signal generated by one male of *Tibicina quadrisignata*.

The alarm signal is produced when the animal is seized or disturbed in the vegetation. Frequently the animal also drops into the shrubs. The oscillogram may be similar to that of the calling song or the time pattern may be irregular, but the damping of the pulses is higher and there is no amplitude increase in the beginning (Fig. 19 C1-2). In the two animals measured, the peak of the average power spectrum was between 8 and 9 kHz (Fig. 20 B) and there was an increase in lower frequency components between 5 and 7 kHz. The -20 dB range relative to peak level seems to be broader than in the calling song extending from about 5 kHz to 16 kHz. The syllable period is slightly prolonged with values of 20 ms at 30 ^{o}C.

Tympanistalna gastrica (Stal, 1854). This species exhibit calling and courtship songs but no alarm signal. I only recorded the calling song. This song consists of trains of sound pulses (Fig. 21-1) delivered periodically (echemes). At irregular intervals, the animals produce three to five shorter echemes (see Fig. 21-3), clearly recognizable by the human ear. Both timbals alternate producing one sound pulse during the inward and another during the outward movement (Fig. 21-5; note the differences in phase between the waves in the beginning of the IN and OUT pulses). The inactivating of one of the timbals halves the number of pulses delivered (Fig. 21-6). The damping of the sound pulses is rather high, resulting in a complete decay of one pulse before the onset of the next one in all but the timbal cycles in the very beginning of the echeme where the sound pulses are closer and the amplitude of the sound pulses, especially the IN, is much higher.

Average periods of the calling song echemes as measured in 11 animals at temperatures between 24 ^{o}C and 26 ^{o}C range from 156 ms (s.d.=4, n=30) to 259 ms (s.d.=7, n=30). Average intervals between echemes were between 21 ms (s.d.=2, n=30) and 42 ms (s.d.=4, n=30) in the same animals. The syllable period is very stable soon after the start of the echeme where it is shorter. The values ranged from 8.3 ms (s.d.=0.4, n=60) to 13.8 ms (s.d.=0.4, n=100). The average power spectrum with a peak frequency within the 10-13 kHz interval and a frequency band extending from 2.5-4 kHz to 15-16.5 kHz (-20dB related to peak) (Fig. 21-5) is rather broad, reflecting the rapid attenuation of the pulses. A second peak is present around 5 kHz. This 5 kHz peak comes from the large pulses occurring at the beginning of the echemes as is demonstrated by an extended sonagram (Fig. 21-4). The subsequent small pulses have similar spectral contents irrespective of being generated during the inward or outward movement of the timbal.

During the courtship song there is a faster cadence of the echemes relative to the calling song and an increase in the generation of echemes of the shorter type. The male becomes silent upon contact with the female and copulation starts immediately.

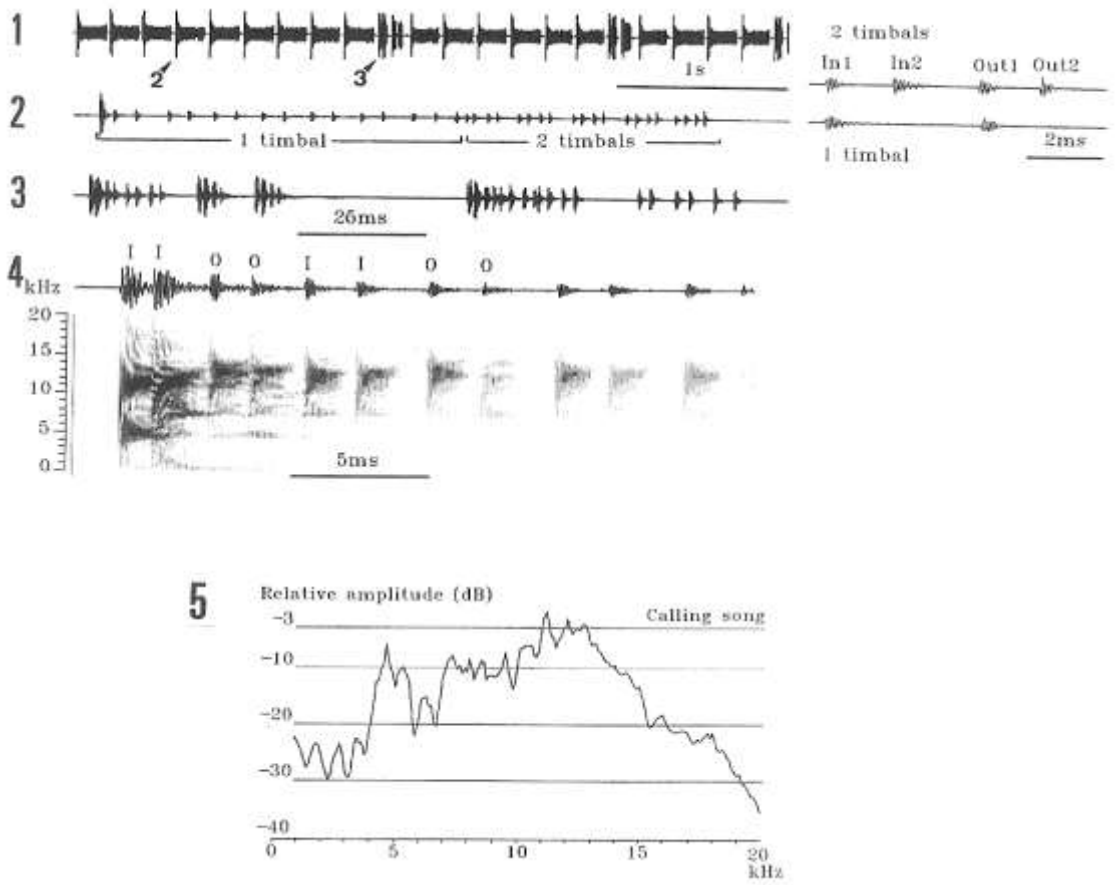


Figure 21 - Calling song of *Tympanistalna gastrica*. 1 - Oscillogram of a sound sequence with different echemes. 2 - Extended oscillogram of a long echeme generated at the beginning with only one timbal but with both timbals producing sound by the end. 3 - Detail of the shorter echemes. 4 - Extended oscillogram and sonagram of the beginning of a long echeme, revealing the characteristic amplitude and frequency modulations of the sound pulses. 5 - Averaged log power spectrum of the calling song.

Discussion. The species examined in this work are good examples of the large variety of sound patterns that can be produced by cicadas with timbals. They also illustrate the importance of the calling song characteristics as an aid to distinguish between morphologically similar species.

A summary of characteristics of the calling songs and alarm signals described above are presented on Table 1.

According to the sound produced during the inward and outward movements of the timbals, these species can be divided into three groups: 1) *Cicada barbara lusitanica* and *C. orni* produce three pulses during the inward movement and one loud pulse during the outward, 2) *Tettigetta argentata* (*atra*), *Tett. estrellae*, *Tett. josei* and *Tettigetta* sp., and *Tympanistalna gastrica* with one pulse in the IN and another in the OUT, and 3) *Tibicina quadrisignata* producing several pulses during the inward (up to seven) related to the buckling of single long ribs of the

timbal, and one faint pulse in the outward movement. Related species of the same genus have timbals that seem morphologically similar and they also seem to have very similar basic mechanics (e.g. similar timbal cycles and spectral contents of songs). This probably reflects their common evolutionary history, but the time pattern, frequency and amplitude modulation patterns of their songs may be clearly different. *Cicada barbara lusitanica* and *C. orni* are a good example as they are very similar morphologically, however the songs differ in the time pattern, which is under the control of the nervous system; the former has a continuous calling song whereas in the latter species it is discontinuous. Also in the Tettigetta species where the timbals produce a single pulse in each of the inward and outward movements, the calling songs are nevertheless very different in their time pattern and amplitude modulation. Both characteristics depend mostly on the nervous system which controls the action and synchronism of the timbals and the action of other structures such as the tensor muscle and abdominal muscles.

In several of the species (*Tett. argentata/atra*, *Tett. josei*, *Tymp. gastrica*) a fast modulation in the amplitude of the sound pulses is observed. *Tett. josei* is a good example. In the calling song of this species the amplitude of the IN pulse can vary greatly in amplitude. In the first of the paired syllables in Part I of the phrase the IN pulse is very quiet and frequently does not appear in the oscillogram, but in the second syllable it has the highest amplitude of all pulses (Fig. 17 A). It seems possible that a change in the stiffness of the timbal may be responsible for those observed changes in amplitude (Pringle 1954). The basis for such a change could be the action of the tensor muscle which inserts on the timbal frame (Pringle 1954).

The uniformity of the pattern of the calling song within a species is likely to be advantageous to secure reproductive success and species isolation. The alarm signals, however, do not meet this constraint; these signals are frequently highly unpatterned (e.g. Haskell 1974; Popov 1975; Popov and Sergeieva 1987). In several species studied here the power spectrum of the alarm signal is broader than that of the calling song, and in particular it has more energy at lower frequencies. I suggest that lower frequencies can be an effective way to communicate the conspecific alarm signals over greater distances in an acoustic environment dominated by the calling song, since in some species the auditory organ is not tuned to the peak of the spectrum of the calling song, but to lower frequencies (see page 258). This mismatch was previously

reported from other cicada species (Popov 1981; Popov et. al. 1985; Popov and Sergeieva 1987). Other reasons for such a difference between the tuning of the auditory organ and the spectrum of the calling song could be the detection of danger sounds, such as those made by predators, in an acoustic environment dominated by the songs of conspecific animals (Popov 1990b). In other species that discrepancy was not found (Simmons et. al. 1971; Young and Hill 1977).

Detailed descriptions of the sound signals are available only for a few European cicadas. Of the species examined in this work, only the song of *C. orni* has been fully described. The present results for this species are basically in accordance with those of Popov (1975), Boulard (1982) and Joermann and Schneider (1987). Some data was also presented by Claridge et. al. (1979). However I did not find the preponderance of echemes with an even number of pulses over those with an odd number, as has been described by Joermann and Schneider (1987). In the alarm signals of *C. orni* analyzed I found the pulse related to the OUT movement of the timbal usually as intense as, or even more intense than the IN pulses. This is different from what was reported by Popov (1975). Also in contrast to what has been observed by the same author in the former USSR, in Portugal several individuals can be observed singing on the same tree or group of trees. Moreover, to my knowledge the courtship song was not described in *C. orni*. However, the modifications on the calling song time parameters in the presence of a flying cicada (or other object) observed by Vogt and Heyl (1992) may correspond to the courtship song in this species.

Boulard (1982) presents sonagrams of the calling songs of *C. barbara lusitanica*, *C. orni*, *Tett. atra*, *Tett. estrellae*, *Tett. josei*, and *Tymp. gastrica*. However the sonagrams do not cover the full spectrum of the calling song. For all species but *C. orni* and *C. barbara lusitanica* the displayed frequency range was below the peak frequency. Also the sonagram presented in Boulard (1990) for the calling song of *Tib. quadrisignata* shows the peak frequency around 3-4 kHz, much below the 8-9 kHz found here. More recently Boulard and Quartau (1991) presented an oscillogram and a sonagram of the calling song of *Tett. estrellae* and described a new species, *Tettigetta septempulsata*. This last species has the same song characteristics described above under *Tett. estrellae* and with the data presented by the authors I am not convinced of the existence of two different species (see arguments on page 12 of "Materials and methods").

Table 1 - Summary of the characteristics of the calling song and alarm signals of some cicada species from Portugal.

Species	Song	Time pattern	Ampl. modulation pattern	Freq. modulation pattern	Timbal relationships (*)	No. pulses /syllable 'in'	No. pulses /syllable 'out'	Syllable period (ms)	No. syllables / echeme	Echeme period (ms)	Power spectrum Peak freq. (kHz)	Power spectrum -20 dB range (kHz)	No. of cicadas analysed
Cicada barbara	Cs	Continuous emission	Regular slow variation at beginning and end	Slow at the beginning	Alternating	(**)	1	8-13	—	—	5-7	2/3-9/11	7
	As	Irregular	Irregular	Irregular	Alternating	3	1	12-15	—	—	3-4		3
Cicada orni	Cs	Regular succession of echemes	Small variations within echemes	Not clear variations	Alternating	(**)	1	8-10	10-30 (variable)		4-5	2-8/11	5
	As	Irregular	Irregular but small	Not clear variations	Alternating	3	1		variable		3-4	1.5/2-6/13	3 (5)?
Tett. argent. /atra	Cs	Regular succession of echemes	Regular fast variation within echemes	Fast within echemes	Alternating or nearly synchronous	1	1	4-6	3	60-90	13-15	5-16	15
	As	Irregular	Irregular	Irregular	Alternating or nearly synchronous	1	1	—	often 4	irregular	13-15	5-16	5 (?)
Tett. estrellae	Cs	Regular succession of complex phrases	Regular slow variation within echemes	Slow within echemes	Close actions	1	1	7-9	variable	variable in complex phrase	11-14	5-16	5
	As	Irregular	Irregular	Irregular	Close actions	1	1	—	variable	irregular	11-14	5-16	3 (5)?
Tett. josef	Cs	succession of complex phrases	Regular fast variation within echemes	—	Nearly synchronous	1	1	—	2 (Part I) 1 (Part II)	22-26 7-10	appr.16	extending to	7
	As	Irregular	Irregular	—	Nearly synchronous	1	1	—	1-4	irregular	—	ultrasonic frequencies	4 (5)?
Tett. sp.	Cs	Succession of complex phrases	Regular fast variation within echemes	Fast within echemes	Alternating	1	1	approx 6	variable	variable (0.1-0.6s)	13-14	6-16	1
Tibicina quadris.	Cs	Continuous emission	Slow regular increase at the beginning	No	Alternating	3-7	1	17-19	—	—	8-10	6-12	5
	As	Irregular	No or irregular	No	Alternating	3-7	1	approx. 20	—	—	8-9	5-16	2 (?)
Tjap. gastrica	Cs	Succession of echemes	Regular fast variation within echemes	Fast within echemes	Alternating	1	1	8-14	variable	150-260 excluding small echemes	10-13	2/4-15/16	11

Cs: Calling song; As: Alarm signal.
(*) Estimated from the sound pulses generated by both timbals.
(**) Difficult to recognize.

I also report the existence of an acoustic male-to-male interaction signal that ceases the courtship of one male to another in *C. barbara lusitanica*. Ceasing courtship by one male to another by means of acoustic signals was also found in any of the six species of Magicicada (Alexander and Moore 1958; Dunning at al 1979; and T.E. Moore, personal communication), but it seems that the signals are less specifically produced.

4. Timbal muscles and sound

Introduction. Despite the similar sound producing apparatus in timballing cicadas, their sound signals differ widely in time, amplitude and frequency patterns. This holds even for signals emitted by the same species (e.g. Popov 1975; Fonseca 1991; see also chapter 3 above). In the past, research on timbal muscle contraction parameters and timbal mechanisms was largely based on EMG recordings during alarm signals (Hagiwara, Uchiyama and Watanabe 1954; Hagiwara 1955; Hagiwara and Ogura 1960; Aidley 1969; Young 1972; Josephson and Young 1981; Young and Josephson 1983a) and the results were usually extrapolated for the calling song. Only in very few cicada species was timbal muscle activity recorded during the generation of calling and courtship songs (Cystosoma saundersii: Simmons 1977, Simmons and Young 1978, Josephson and Young 1979; Magicicada cassini and M. septendecim: Reid 1971, Young and Josephson 1983b, Weber et. al. 1987; Okanagana vanduzeei: Josephson and Young 1985).

In order to allow the above mentioned extrapolations it is necessary to investigate the similarity of the motor pattern employed during the generation of the calling song and the alarm signal. In order to compare the two patterns the output of each timbal action both during calling songs and alarm signals was observed. Secondly, a number of parameters were measured such as the duration of the cycle of the timbal muscle contraction, the left-right interval of the timbal muscles contraction (intertimbal delay) and their phase relationships, and changes on the leading muscle during singing. Such data allows to determine accurately the similarities and the differences in timbal muscle activity and sound pulses between calling song and alarm signal.

The high degree in sound amplitude modulation common in cicada songs also rases questions: Are these modulations caused by the timbal muscle activity alone, or is there evidence for an influence of other mechanical systems? It is conceivable that these sound amplitude modifications could be dued to the timbal muscle itself, for instance by a change of the contraction rate. Other possible mechanisms involve either the timbal tensor muscle which might change the timbal stiffness (Pringle 1954) or other structures involved in sound radiation such as the abdomen (Simmons and Young 1978; Fletcher and Hill 1978) or the tympana (Weber et. al. 1987; Young 1990). The following measurements would allow to distinguish between these possibilities:

A) A correlated change of the timbal muscle contraction rate and the sound pulse amplitude. B) Correlated changes in sound amplitude and time lag from the timbal muscle EMG to the IN sound pulse may indicate a modified timbal stiffness, as reported by Pringle (1954) and Simmons and Young (1978). Furthermore, the modifications of the time interval between IN and OUT pulses would also indicate changes in the elastic restoring force of the timbal and/or relaxation properties of the timbal muscle. C) Changes only in amplitude without changes in the time lag might indicate that the amplitude of sound radiation is influenced by the abdomen and/or tympana and opercula.

This work attempts to contribute to these two major questions. The results are based on simultaneous recordings of sound and timbal muscle EMGs during the calling song and the alarm signal obtained from six cicada species.

Results.

Analysis of calling songs recorded in the field and in the laboratory. The species studied here produced their calling songs in the laboratory while tethered and with electrodes implanted in order to record the EMG from their timbal muscles. Table 2 compares the ranges of the timbal muscle contraction frequencies during the calling song derived from the EMG recordings in the laboratory and the calculated contraction frequencies for the calling songs recorded in the field (calculated from data presented on chapter 3). The patterns of the songs and the timbal muscle contraction frequencies were similar to the ones observed in the field (Table 2).

Because of the differences in the song patterns and in the timbal mechanisms found in the several species studied here, the results for each species are presented separately. Moreover, the species were grouped according to similarities on their results.

Table 2 - Comparison between the calculated rates of timbal muscle contraction on the calling songs recorded in the field and on the calling songs produced under laboratory conditions with EMG electrodes implanted in the timbal muscles.

Species	Calling song (field)	Calling song (Laboratory)
Tettigetta argentata	130-240 Hz	125-250 Hz
Tettigetta josei	100-140 Hz	80-170 Hz
Tympanistalna gástrica	70-120 Hz	60-110 Hz
Cicada barbara lusitanica	80-120 Hz	95-105 Hz
Tibicina quadrisignata	53-57 Hz	49-58 Hz

Tettigetta argentata/atra. *Tett. argentata/atra* generates one sound pulse when the timbal buckles inward (IN) and one when the timbal buckles outward (OUT). During the calling song this species shows a very regular sequence of echemes (Fig. 22 A) each of which consists of three pairs of IN and OUT sound pulses (Fig. 22 A1,2). The time pattern of the echemes was very stable during the calling song where the echeme period became constant a short time after the animals started calling. In contrast the echeme period was irregular in the alarm signal (cf. Fig. 22 A and B).

During the calling song the IN sound pulse was very quiet in the beginning of each echeme but most intense by the end (Fig. 22 A2). In contrast, in the alarm signal (Fig. 22 B) the IN sound pulse was often similar throughout the echeme and less intense (or at about the same intensity) than the OUT pulse. A similar situation could occur when an animal started its calling song (Fig. 22 A1).

During the calling song each timbal muscle contracted 3 times per echeme (Fig. 22 A1,2), but in the alarm signal it was often found 4 actions per echeme with an observed range from 1 to 5 actions per echeme (Fig. 22 B).

The cycle of the timbal muscle contractions might be longer in the alarm (5.5-10 ms) than in the calling song (4-8 ms) (Fig. 22 C). The period of the EMG increased throughout the echeme in the calling song and often also in the alarm signal (Fig. 22 C). However this increase was very regular in the calling song but most variable in the alarm.

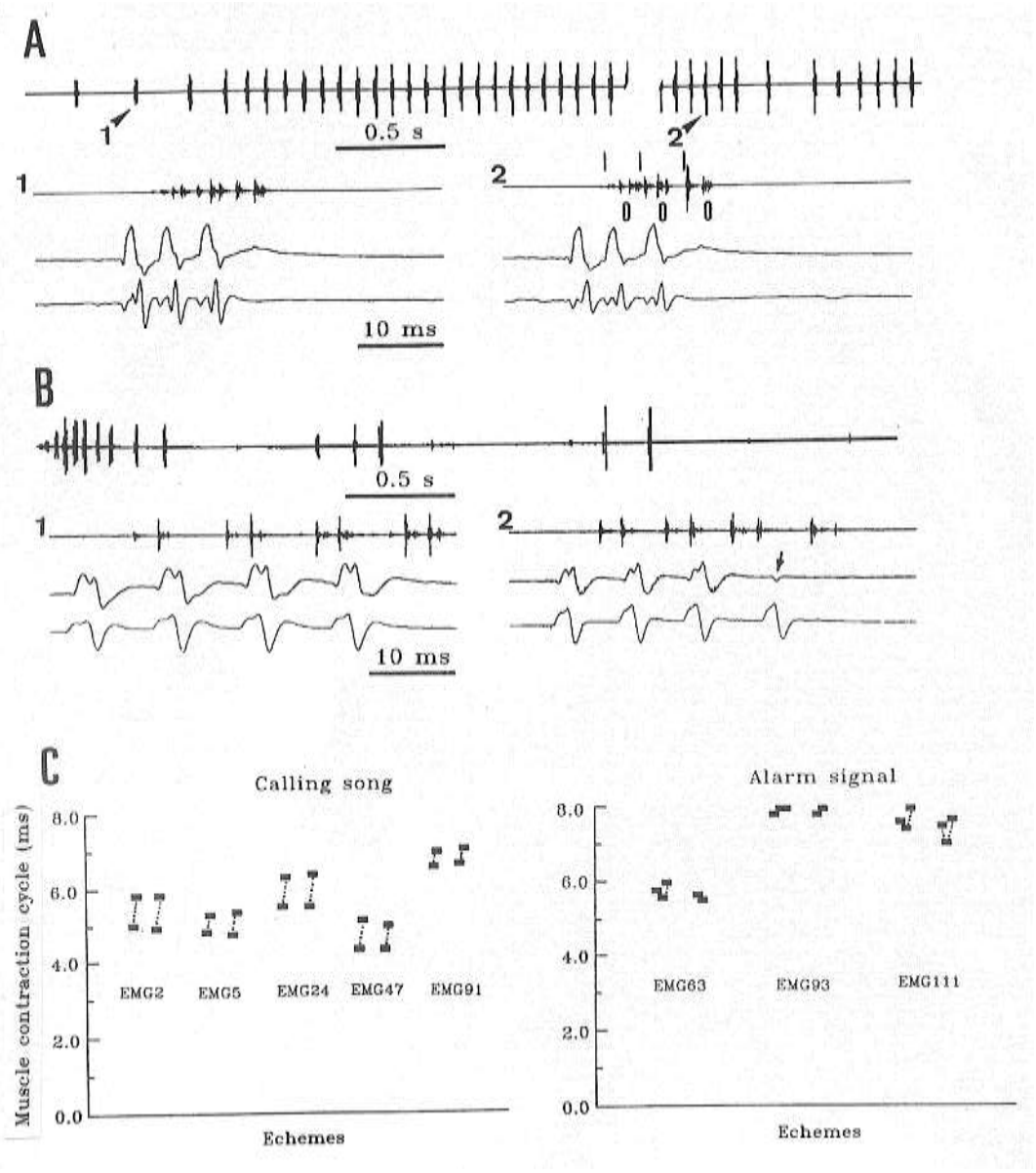


Figure 22 - Sound signals and timbal muscles EMG of *Tettigetta argentata/atra*. A) Calling song sequence showing (1) an echeme in the beginning of the song and another one recorded later (2) displaying the normal amplitude modulation pattern. B) The alarm signal is irregular in time; 1, 2 expanded sections. Note the small artifact caused by the cross-talk between both EMG electrodes shown when one timbal muscle failed to contract (arrow on B2). Despite of regular timbal muscle EMG patterns there are large modifications in the sound amplitude, especially in the calling song. I=IN; O=OUT. C) The change in the duration of the timbal muscle contraction cycle within an echeme. Two echemes of each of 5 and 3 males generating calling and alarm signals, respectively, are presented.

During the production of both signals the intertimbal delay remained relatively constant. The intertimbal delay in the calling song of three animals was 0.7-0.9 ms, 0.8-1.0 ms and 1.2-1.4 ms. In the alarm signal of one animal it was constant around 0.6 ms but less constant in a second one where sometimes in the beginning of the echeme the muscles seemed to contract simultaneously, and then kept a fixed time lag of 0.7-0.8 ms. Despite of the maintained left-right delay exhibited by the EMGs, the last IN sound pulses of the calling song echemes were often produced almost synchronously (Fig. 22 A2). The phase of the bilateral muscle contraction was

around 0.1 in both sound signals and less stable than the intertimbal delay. The timbal muscle which was active first in an echeme always remained the leading muscle in all echemes generated throughout the calling song. During the alarm signal the leading muscle was also not changed.

The time lag from the timbal EMG to the IN sound pulse during the calling song increased throughout the echeme (100% longer). There was a positive correlation between the IN sound pulse amplitude and the time lag EMG-IN in the calling song (Fig. 23 A). The OUT amplitude appeared to be independent of the time lag EMG-IN (Fig. 23 B). The increase in amplitude was accompanied by an increase in the time lag from the muscle potential to the IN sound pulse, but was not correlated with changes in the EMG of the timbal muscles. In contrast with the calling song, the time lag EMG-IN in the alarm signal was stable or even reduced towards the end of the echeme, and a correlation between the IN sound pulse amplitude and the time lag EMG-IN did not seem to occur.

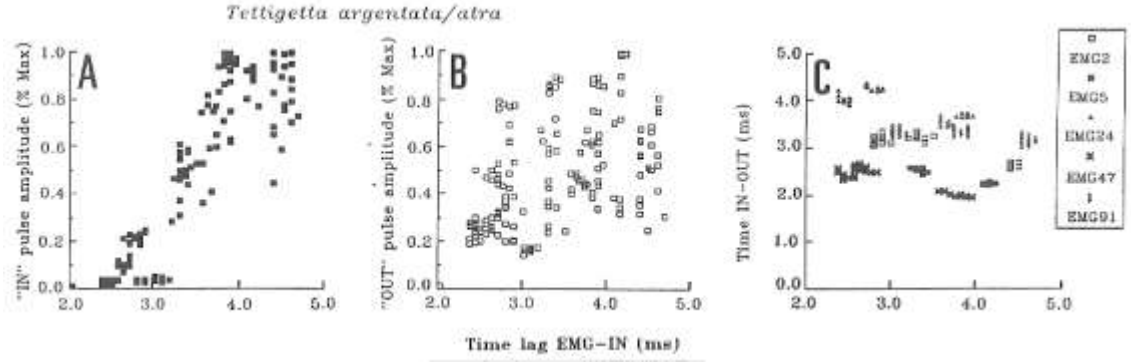


Figure 23 - Relationships between the time lag from the timbal EMG to the IN pulse and some sound pulse parameters measured in the calling song of *Tettigetta argentata/atra* (5 animals). A and B) Relation between the sound pulses amplitude (IN and OUT) and the time lag EMG-IN. The IN sound pulse amplitude is positively correlated with the time lag EMG-IN while the OUT sound pulse seems to be independent. C) Relationship between the time interval measured between the sound pulses produced by the inward and subsequent outward movements of the timbal, and the time lag EMG-IN. A negative correlation occurs considering the second and third syllables of the calling song echemes.

During the calling song the time between IN and OUT pulses decreased in the last IN-OUT pulses of the echeme (for one timbal it seemed to be shorter than for the other). The time lag EMG to IN was inversely related to the time IN-OUT (Fig. 23 C), but this relation was not linear especially in the calling song. The time between IN-OUT sound pulses often increased towards the end of the echeme in the alarm signal.

Tettigetta josei. *Tett. josei* also generates one IN and one OUT sound pulse when the timbal buckles in and out. The calling song consists of a succession of phrases. Each phrase is composed by a regular series of echemes each of which consists of two pairs of IN and OUT sound pulses (Fig. 24 A1,2) during Part I, and then a series of single pairs (Part II). The time pattern during the calling song was complex (Part I, Part II) but regular. In contrast it was irregular during the alarm signal (cf. Fig. 24).

In the calling song of this species (Part I) the first IN sound pulse in the beginning of each echeme was very quiet (sometimes not apparent at all), whereas the second one was the most intense (Fig. 24 A2). The OUT sound pulses remained constant in amplitude throughout the song. However, in the beginning of Part I the IN pulse of the second syllable was initially soft, similar to the first one (Fig. 24 A1), and later increased in intensity to reach the characteristic maximum (see graph inset on Fig. 24 A). During Part II the IN was always intense and louder than the OUT pulse (Fig. 24 A3). In contrast, during the alarm signal (Fig. 24 B) there was no definite pattern in the amplitude modulation of the echemes, and the IN sound pulse could be loud or soft, but usually it was not much louder than the OUT pulse.

During the calling song the timbal muscles contracted 2 times per echeme (Part I) or only once in a regular series of contractions (Part II). However, in the alarm signal three muscle actions per echeme were often found, and it was possible to see from one to four contractions.

The cycle of the timbal muscle contractions was more stable in the calling song than in the alarm signal. The average cycle of the timbal muscle contractions within the echemes ranged 7-10 ms (s.d.<0.2) in the Part I (excluding the very beginning) in the 5 animals where the calling song was measured. In the alarm signal the cycles were 7.8 ms (s.d.=0.75) and 8.5 ms (s.d.=1.26). In the beginning of each phrase in the calling song the cycle of timbal muscle contraction within the echemes increased to reach a stable value (see Fig. 24 C). In the very beginning the second contraction came sometimes so early (cycle about 5 ms) that the muscle seemed to have no time for relaxation and the OUT pulse was delayed by about the cycle length. In Part II the time structure with 2 contractions per echeme changed to a sequence of single contractions (Fig. 24 A3). The cycle of timbal muscle contraction first increased to nearly the

double of the normal cycle within the echemes, and then decreased approaching the normal value when the phrase stopped (Fig. 24 C).

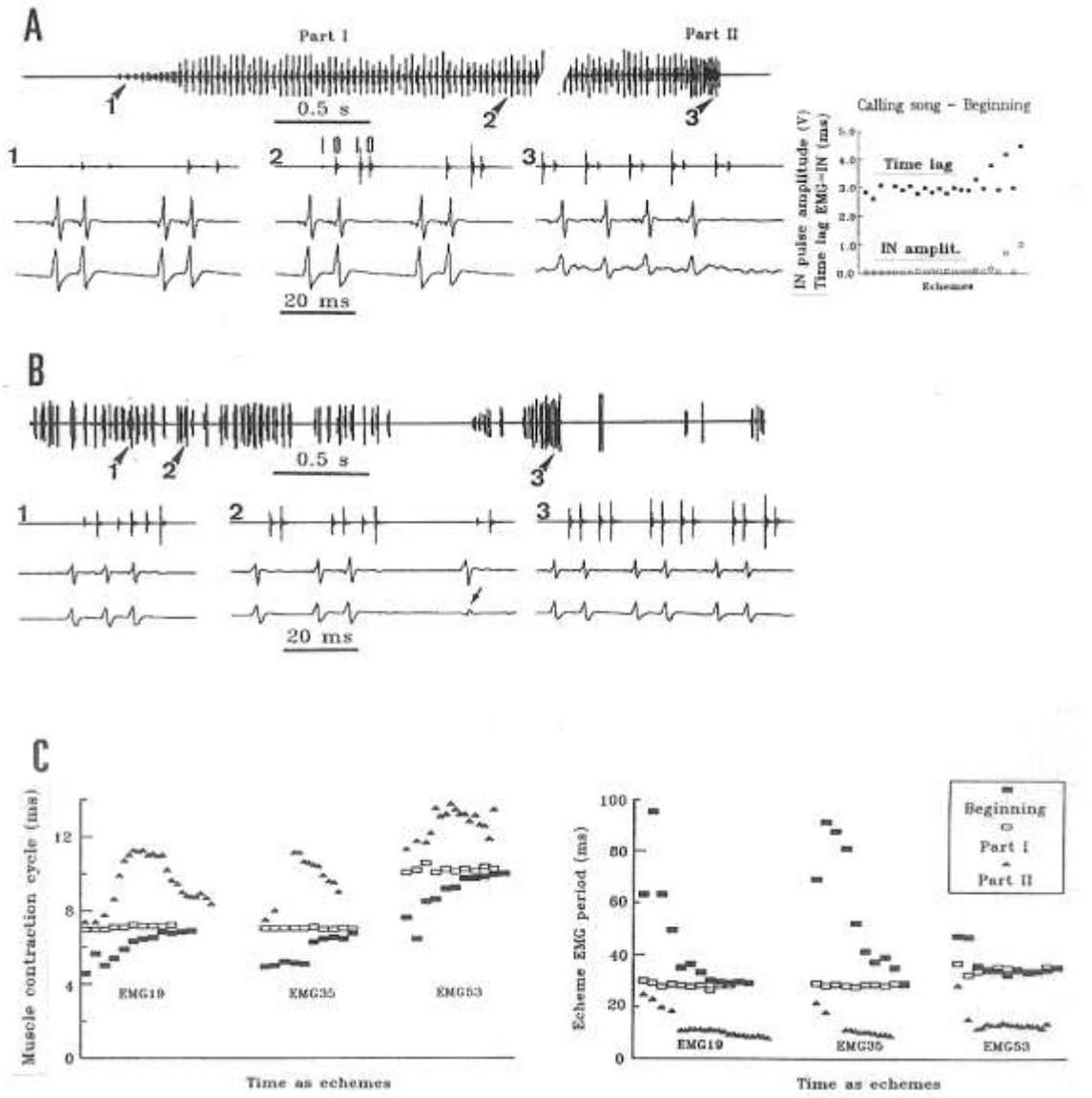


Figure 24 - Sound signals and timbal muscles EMG of *Tettigetta josei*. A) Calling song sequence showing Part I and Part II. (1) Two echemes in the beginning of a phrase of the song, (2) two echemes in the middle displaying large amplitude changes in the IN sound pulse and (3) the end of the phrase where the IN sound pulse amplitude is always large. The inset graph shows the changes of the IN sound pulse amplitude and the time lag EMG-IN which occur at the beginning of the sound sequences. B) The alarm signal is irregular. The timbal muscles act almost synchronous. Note the small artifact caused by the cross-talk between both EMG electrodes shown when one timbal muscle failed to contract (arrow on B2). I=IN; O=OUT. C) Changes in the duration of the timbal muscle contraction cycle and in the echeme period as measured in the timbal muscles EMG. At the beginning, in the middle (Part I) and at the end (Part II) of the calling song phrase there are the same consistent changes for all three animals.

At the beginning of each phrase the echeme period was larger, rapidly decreasing to stabilise around 30 ms (Fig. 24 C).

Tett. josei is an example of a species presenting timbal synchronization. The intertimbal delay remained constant in the calling song. The intertimbal delay measured in two animals was about 0.3 ms (s.d.=0.04 and 0.03, n=10). In the alarm signal it was less constant and I measured values of 0.4 ms (s.d.=0.25, n=10) and 0.6 ms (s.d.=0.12, n=10) in another two animals. The phase of the bilateral muscle contraction was very stable in the calling song (2 animals) with values of 0.02-0.04 and negligible standard deviation. In the alarm signals of another two animals the values obtained were 0.05 (s.d.=0.03) and 0.08 (s.d.=0.02). There was not any case of changing in the leading muscle detected during the calling song. In the alarm signal, one of the two animals recorded shifted frequently the leading muscle, especially at the beginning of the echemes.

The time lag EMG-IN sound pulse was variable and related to the IN pulse amplitude and to the time between IN-OUT. In both songs there was a positive correlation between the IN pulse amplitude and the time lag EMG-IN (Fig. 25 A). The IN sound pulse amplitude in Part I of the calling song increased from virtually nothing to the maximum and was accompanied by a 200% increase in the time lag EMG-IN. While there were large changes in time lag and amplitude, no according changes were seen in the timbal muscles EMG. The amplitude of the OUT pulse was independent of the time lag EMG-IN (Fig. 25 B). There was a negative correlation between the time IN-OUT and the time lag EMG-IN (Fig. 25 C).

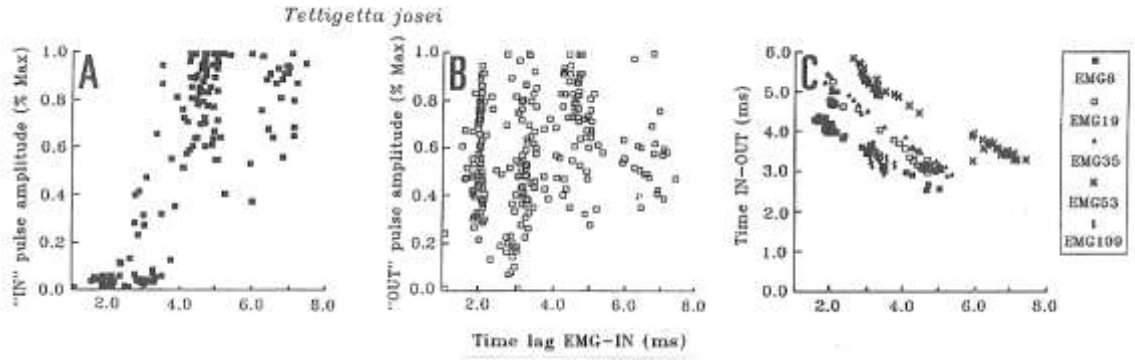


Figure 25 - Relationships between the time lag from the timbal EMG to the IN pulse and some sound pulse parameters measured in the calling song of *Tettigetta josei* (4 animals). A and B) Relation between the sound pulses amplitude (IN and OUT) and the time lag EMG-IN. The IN sound pulse amplitude is positively correlated with the time lag EMG-IN while the OUT sound pulse seems to be independent. C) Relationship between the time interval measured between the sound pulses produced by the inward and subsequent outward movements of the timbal, and the time lag EMG-IN. A negative correlation occurs.

In the beginning of the phrases there was often a lack of the characteristic modulation of the IN sound pulse, accompanied by a constant time lag EMG-IN. Suddenly, without changes in the

timbal muscles EMG, the amplitude modulation and time shifts started slowly to develop until the normal pattern was attained.

Tympanistalna gastrica. In this species there was usually no alarm signal.

Tymp. gastrica generates one IN and one OUT sound pulse when the timbal buckles in and out. The calling song consists of a succession of echemes (Fig. 26 A) which may be included in three different types. The most frequent one (Type 1 - T1) was much longer then the 2 other kinds: Type 2 (T2) and Type 3 (T3), which resulted of usually only 3-4 timbal muscle contractions.

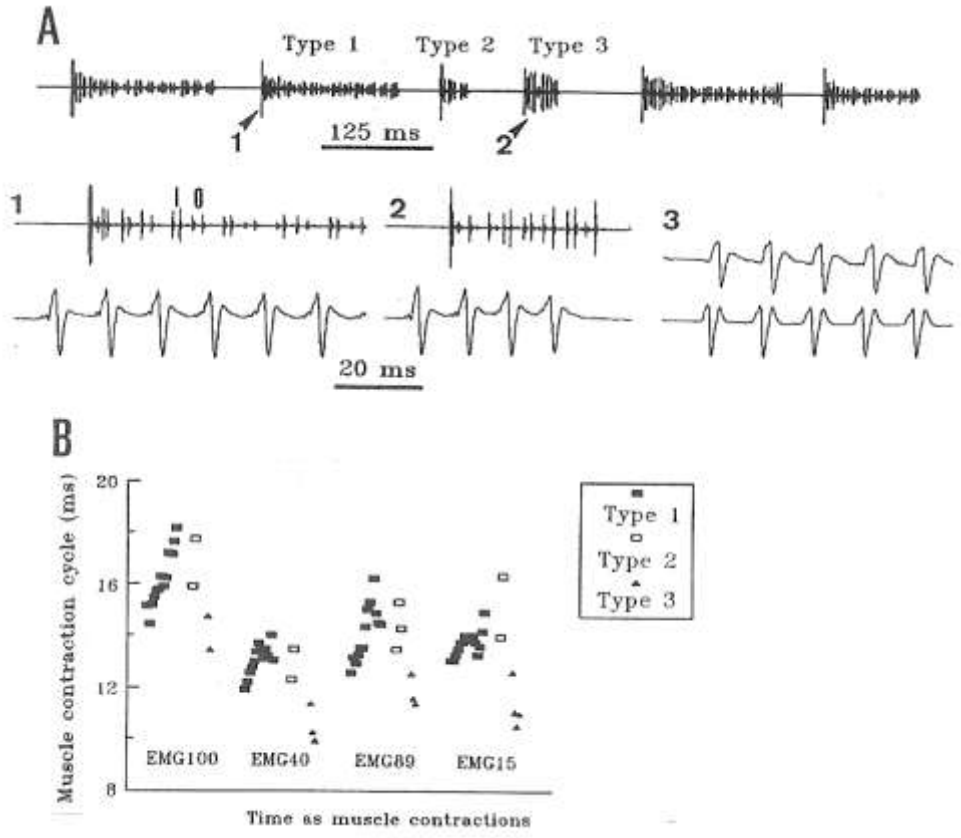


Figure 26 - Calling song and timbal muscles EMG of the cicada *Tympanistalna gastrica*. A) There are 3 types of echemes. (1) the beginning of a "Type 1" echeme and (2) of a "Type 3" echeme. (3) Soundless recording showing the activity of both timbal muscles. I=IN; O=OUT. B) Changes of the duration of the timbal muscle contraction cycle observed on the several echemes found in the calling song of four males.

The IN and OUT sound pulses were more or less similar in amplitude except in the beginning of the echemes (sometimes not in T3). The first bilateral IN sound pulses (sometimes merging together) were larger than the OUT and much larger than the subsequent ones (Fig. 26 A).

The average cycle of the timbal muscle contractions ranged 9-18 ms in 6 animals measured. There was a clear pattern in the cycle of timbal muscle contraction that was different

in the different kinds of echemes (Fig. 26 B). On echemes T1 and T2 the muscle cycle increased in the beginning. While in some cases it grew throughout all the echeme, in other cases it either stabilised or decreased by the end of the echeme. In contrast, on echemes T3 the cycle decreased throughout the short echeme reaching the shortest values found in the song (in some cases there was no sound produced by the last muscle contraction). The sound amplitude seemed to be independent of the muscle contraction cycle. The muscle contraction cycle seemed to decrease during continuous sound production.

During the calling song the intertimbal delay remained relatively constant. The intertimbal delay ranged from 2.1 ms (s.d.=0.04) to 3.0 ms (s.d.=0.11) in four animals. In the beginning of the echemes the intertimbal delay was usually slightly shorter.

The phase of the bilateral muscle contraction was also relatively stable in each recording. Average values ranged in the same four animals from 0.11 (s.d.=0.01) to 0.22 (s.d.=0.01). The leading muscle never changed during the calling song.

The time lag from the timbal muscle potential to the IN sound pulse was variable and was related to the IN pulse amplitude and to the time between IN and OUT (Fig. 27 A,C). There was a positive correlation between the time lag EMG-IN and the amplitude of the IN sound pulse on echemes T1 and T2 (Fig. 27 A). This relationship was not as clear on echemes T3 (cf. Fig. 27 A). From the beginning of the echemes (T1 and T2) starting with the large pulses, there was a subsequent reduction of the time lag to about half of the value, accompanied by a great reduction of the IN pulse amplitude. There was a jump on the time lag from values around 10-12 ms to values about 5-6 ms and a corresponding discontinuity in the amplitude (Fig. 26 A1,2; Fig. 27 A). On echemes T3 the IN and OUT pulses were usually larger than the ones in the other echemes for the same shorter time lags. It was possible that a second and different mechanism was implied in generating these larger sound pulses.

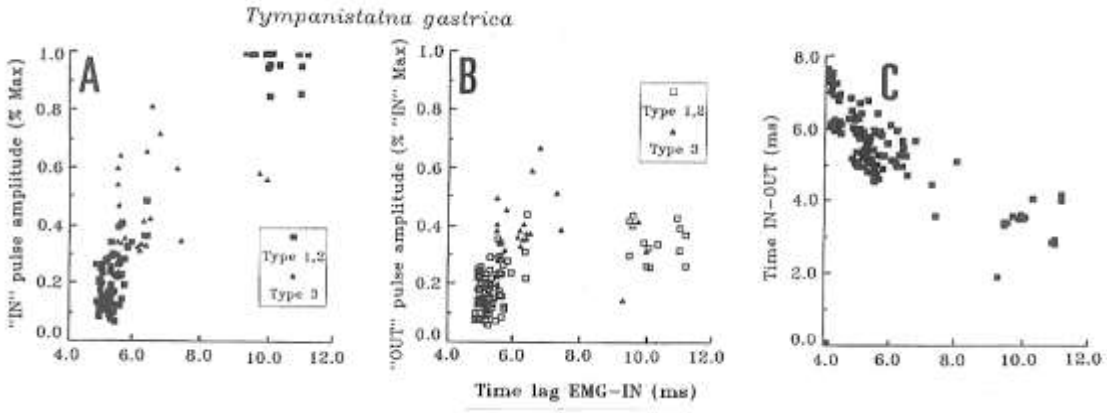


Figure 27 - Relationships between the time lag from the timbal muscle potential (EMG) to the IN pulse and some sound pulse parameters measured in the calling song of *Tympanistalna gastrica* (5 animals). A and B) Relation between the sound pulses amplitude (IN and OUT) and the time lag EMG-IN. The IN sound pulse amplitude is positively correlated with the time lag EMG-IN on echemes of types 1 and 2, but this relation is not clear on Type 3. C) Relationship between the time interval measured between the sound pulses produced by the inward and subsequent outward movements of the timbal, and the time lag EMG-IN. A negative correlation occurs.

The amplitude of the OUT pulse was also larger for larger EMG-IN time lags but the correlation was less clear (Fig. 27 B). There was a negative correlation between the time IN-OUT and the time lag EMG-IN (Fig. 27 C).

Cicada barbara lusitanica. In this species there were usually three sound pulses corresponding to one inward movement of the timbal while the outward movement generated only one sound pulse. At the beginning of each calling song (and courtship song) there was a gradual increase in the sound amplitude until a maximum was reached (Fig. 28 A). At the end of the long calling sequences the amplitude usually decreased. These amplitude modulations were missing in the alarm signal (Fig. 28 B). The variations in the sound amplitude were more emphasised in the IN pulses than in the OUT sound pulse.

In the loud region of the calling song (and courtship song) the IN sound pulses had a higher amplitude than the OUT pulse (Fig. 28 A2). In contrast, during the alarm signals the OUT pulse was usually louder than the IN pulses (Fig. 28 B1,2). This situation also occurred in the beginning of the calling and courtship songs (Fig. 28 A1). During the gradual sound amplitude increase in the calling (and courtship) the amplitude of the IN sound pulses increased much more than that of the OUT pulses (cf. Fig. 28 A1 and A2).

The sound sequences were usually much shorter and irregular in the alarm than in the calling song (Fig. 28 A and B). It was frequent to find absences of contraction of one of the timbal muscles during the generation of alarm signals, a situation which was rare in the calling song.

The cycles of the timbal muscles contraction were variable throughout the calling and courtship songs (see Table 3). The values decreased at the beginning of the sound sequences (11-22% in 3 animals), while the sound amplitude strongly increased (the IN sound pulses can increase 4-8 times and the OUT can be more than double in a free singing cicada). By the end of the sequences the muscle contraction cycle became much longer (64-115% in 3 animals). However this change was not accompanied by a pronounced decrease in loudness similar to the amplitude increase at the beginning of the calling song. Mechanisms other than the muscle contraction rate must be responsible by the amplitude modulation (see also Fig. 28 C).

The muscle contraction cycle in the alarm signals was usually similar to the shorter cycles of the calling and courtship. Values in 4 animals were: 9.6 (s.d.=0.3); 11.6 ms (s.d.=2.4), 13.5 ms (s.d.=3.0) in two different recordings; 12.2 (s.d.=1.1); 9.5 (s.d.=0.4) and 14.6 (s.d.=2.4) in two different recordings.

Table 3 - Cycle of the timbal muscles contraction in the calling song of *Cicada barbara lusitanica*.

Song	Beginning	Middle	End of sequence
CS	----	13.2 (s.d.=0.9)	21.6 (s.d.=4.8)
CS	----	10.8 (s.d.=0.6)	approx.29
CS	13.6 (s.d.=3.5)	10.5 (s.d.=0.7)	21.4 (s.d.=1.7)
CS*	11.0 (s.d.=1.7)	9.4 (s.d.=0.1)	20.2 (s.d.=3.8)
Court.*	13.1 (s.d.=1.1)	10.8 (s.d.=0.1)	21.2 (s.d.=0.6)

* Estimated from the song.

The intertimbal delay was variable in all sound signals. This variation was gradual in the calling and courtship songs. The intertimbal delay was larger in the beginning and again at the end of the sequences, and the smaller values were found at higher sound amplitudes (see Table 4 and cf. Fig. 28 A1, A2 and A3). The reduction in the intertimbal delay occurring at the beginning of the calling sequences ranged 4-20% while the increase observed at the end of the sequences reached values from 50% to 90%.

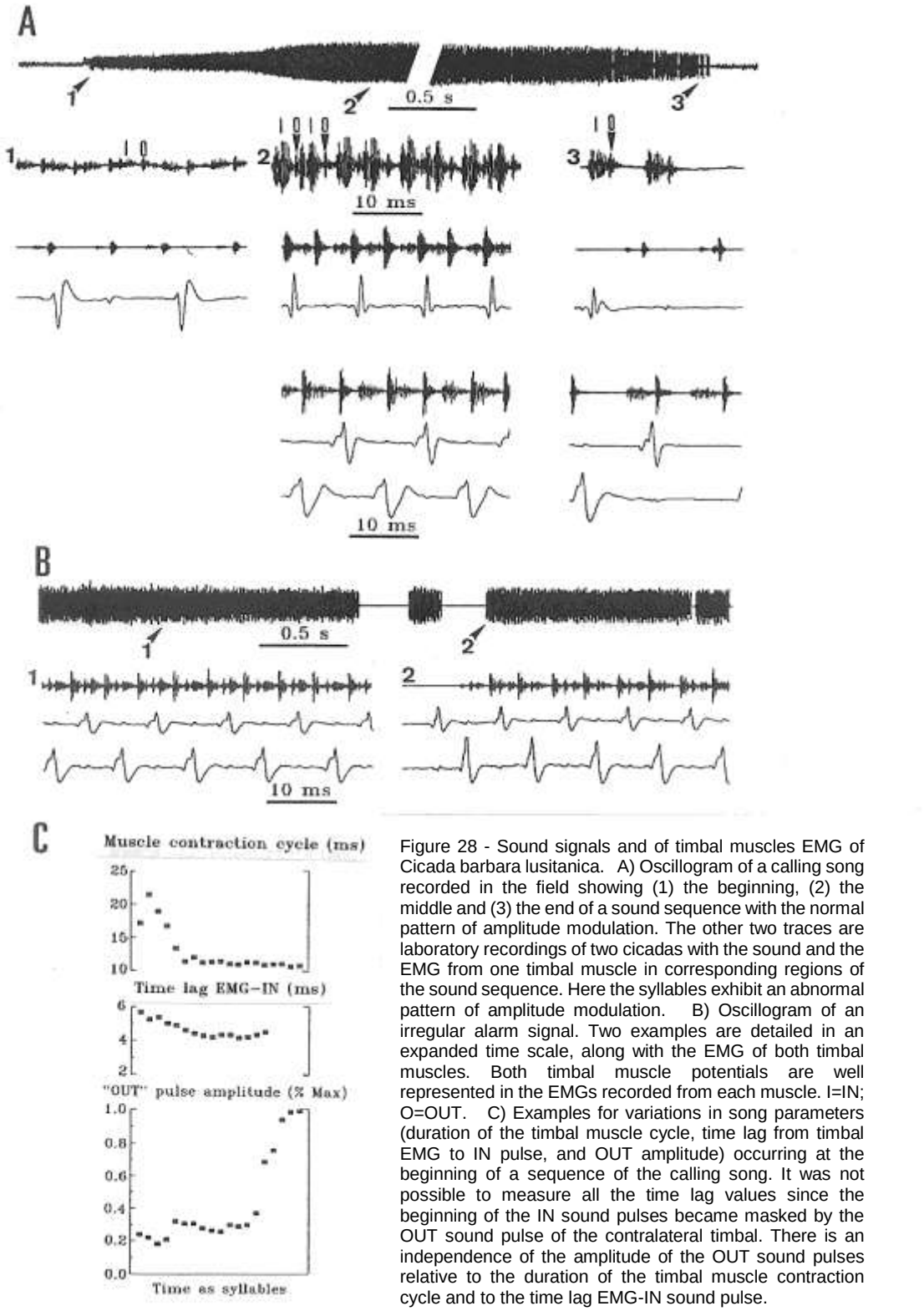


Figure 28 - Sound signals and of timbal muscles EMG of Cicada barbara lusitanica. A) Oscillogram of a calling song recorded in the field showing (1) the beginning, (2) the middle and (3) the end of a sound sequence with the normal pattern of amplitude modulation. The other two traces are laboratory recordings of two cicadas with the sound and the EMG from one timbal muscle in corresponding regions of the sound sequence. Here the syllables exhibit an abnormal pattern of amplitude modulation. B) Oscillogram of an irregular alarm signal. Two examples are detailed in an expanded time scale, along with the EMG of both timbal muscles. Both timbal muscle potentials are well represented in the EMGs recorded from each muscle. I=IN; O=OUT. C) Examples for variations in song parameters (duration of the timbal muscle cycle, time lag from timbal EMG to IN pulse, and OUT amplitude) occurring at the beginning of a sequence of the calling song. It was not possible to measure all the time lag values since the beginning of the IN sound pulses became masked by the OUT sound pulse of the contralateral timbal. There is an independence of the amplitude of the OUT sound pulses relative to the duration of the timbal muscle contraction cycle and to the time lag EMG-IN sound pulse.

The intertimbal delay in the alarm signals measured in 4 animals was: 6.3 ms (s.d.=0.6), 6.5 ms (s.d.=1.7), 14.7 ms (s.d.=0.2), and in two signals of a 4th animal the values were 4.7 ms (s.d.=0.3) and 7.9 ms (s.d.=2.2).

The phase of the left-right muscle contraction was usually around 0.5 (0.48-0.55), despite the irregularity in the muscle contraction cycle. It was more stable in the calling than in the alarm signals. At the end of the long calling song sequences the phase values might decrease considerably (the extreme value was 0.31) since the longer muscle contraction cycles were not completely compensated by the increase in the intertimbal delay. The leading muscle might change in both signals (calling and alarm). The changes, however, were not detected in the middle of the sound sequences.

Table 4 - Intertimbal delay in the calling song of *C. barbara lusitanica*.

Song	Beginning	Middle	End of sequence
CS	----	6.6 (s.d.=0.5)	9.9 (s.d.=1.1)
CS	----	5.4 (s.d.=0.4)	10.1 (s.d.=0.7)
CS	7.0 (s.d.=1.9)	5.8 (s.d.=0.4)	10.7 (s.d.=1.4)
CS*	5.3 (s.d.=1.1)	5.1 (s.d.=0.1)	7.9 (s.d.=0.6)
Court.*	6.9 (s.d.=0.7)	5.5 (s.d.=0.1)	10.1 (s.d.=0.1)

* Estimated from the song.

The time lag from the timbal muscle potential to the IN sound pulse was relatively stable during each sound sequence. However the time lag might change considerably in the same animal. Values ranged in the two kinds of signals from 2.9 ms to 8.3 ms in 4 animals (calling song: 4.1-8.3 ms, alarm signal: 2.9-6.5 ms). The alarm signals showed similar or shorter time lags than the calling song. A clear dependence of the sound pulse amplitude on the time lag EMG-IN, as found in the other species (Figs. 23 A, 25 A, 27 A), was not seen (cf. Fig. 28 C). In some recordings it was not possible to measure this time lag because the OUT pulse of one timbal masked the IN of the contralateral timbal.

Cicada orni. In this species I only recorded the timbal muscles EMG during the production of the irregular alarm signal (Fig. 29) since I was not successful in elicit singing in the laboratory.

The timbal cycle found in this species is basically similar to the one of *C. barbara lusitanica*. There were usually 2-3 sound pulses corresponding to each inward movement of the timbal. The outward movement generated only one sound pulse. The IN sound pulses might be louder or softer than the OUT pulse. The OUT pulse of one timbal usually masked the IN sound pulse of the contralateral timbal. However, the signals of *C. orni* have not a marked sound amplitude modulation.

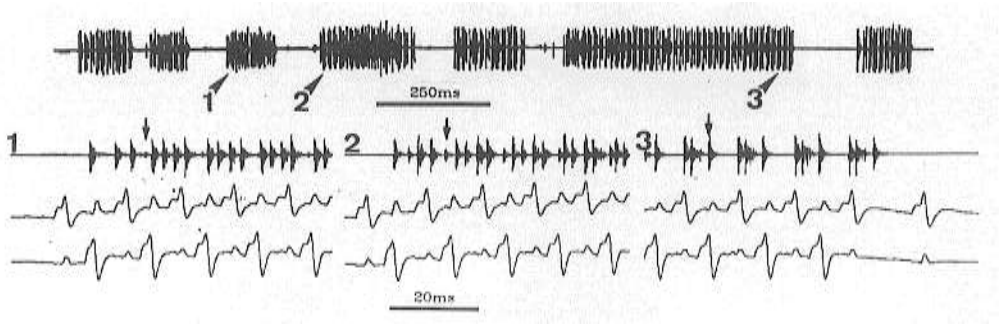


Figure 29 - Alarm signal and timbal muscles EMG of *Cicada orni*. From 1 to 3 there is an increase in the OUT pulse amplitude, indicated by arrows, without any noticeable change in the timbal muscles EMG. Contrasting with 1 and 2, on 3 only one timbal is producing sound.

The muscle contraction cycle ranged from 9.6 ms (s.d.=0.15) to 14.6 ms (s.d.=1.02).

The intertimbal delay was variable and might change in the same animal. Values measured in 5 animals ranged from 4.9 ms (s.d.=0.10) to 7.9 ms (s.d.=0.76).

The phase of the left-right muscle contraction was usually between 0.50 and 0.55. The phase was less stable at the beginning and at the end of the alarm sequences.

The time lag EMG-IN was relatively stable in each sound sequence. However it might change over time in the same animal. Values in 5 animals ranged 4-7 ms. It was not seen a clear positive correlation between the pulse amplitude and the time lag EMG-IN. Changes of the leading muscle were observed. The animals could start the sound sequences with one or the other timbal muscles.

Tibicina quadrisignata. *Tib. quadrisignata* generates up to seven IN and one very soft OUT pulse when the timbal buckles in and out. During the inward movement of the timbal there is a one-to-one correspondence between the buckling of the timbal ribs and the sound pulses produced. At the beginning of the long sound sequences in the calling song (and courtship song) there was an

increase in the sound amplitude until a steady maximum was reached (amplitude increase ranging from 1.5 to 5 times in 7 animals). This amplitude modulation was not present in the alarm signal (compare Fig. 30 A and B).

The number of timbal ribs involved in sound production increased in the beginning of each sound sequence in the calling song (and courtship song). The number of ribs involved, from 2-4 in the beginning to a stable 6-7, did not seem to be dependent on the syllable period (muscle contraction cycle). From the 7 animals measured there was only one case where an increase of the number of ribs clicking inward was accompanied by a decrease in the syllable period. In the other 6 animals the period was relatively stable.

The sound sequences were much shorter and irregular in the alarm signal than in the calling song (Fig. 30 A,B). It was also frequent to find absences of contraction of one of the timbal muscles in the alarm signal, a situation that was rare in the calling song.

The cycle of the timbal muscle contraction was similar in the calling song and in the alarm signal, averaging from 17.5 ms to 22 ms (6 animals). It was however more variable in the alarm signal and in the beginning and at the end of the sequences of the calling song. The increase in the sound amplitude in the beginning of the calling song sequences was not correlated with the rate of the timbal muscle contraction (Fig. 30 C).

In the calling song the intertimbal delay remained constant during all but the very beginning of the sound sequences. The intertimbal delay increased 10%-20% in the beginning of each sequence. The variations measured in three animals were from 6.8 ms (s.d.=0.52) to 7.8 ms (s.d.=0.08), from 5.4 ms (s.d.=0.26) to 5.9 ms (s.d.=0.18), and from 5.8 ms (s.d.=0.21) to 6.8 ms (s.d.=0.10). In the alarm signal the values were less constant and also larger than in the calling song. The time measured between the potentials of the two timbal muscles in four animals was 9.2 ms (s.d.=0.68), 9.3 ms (s.d.=0.61), 11.1 ms (s.d.=3.56), and 11.1 ms (s.d.=1.95).

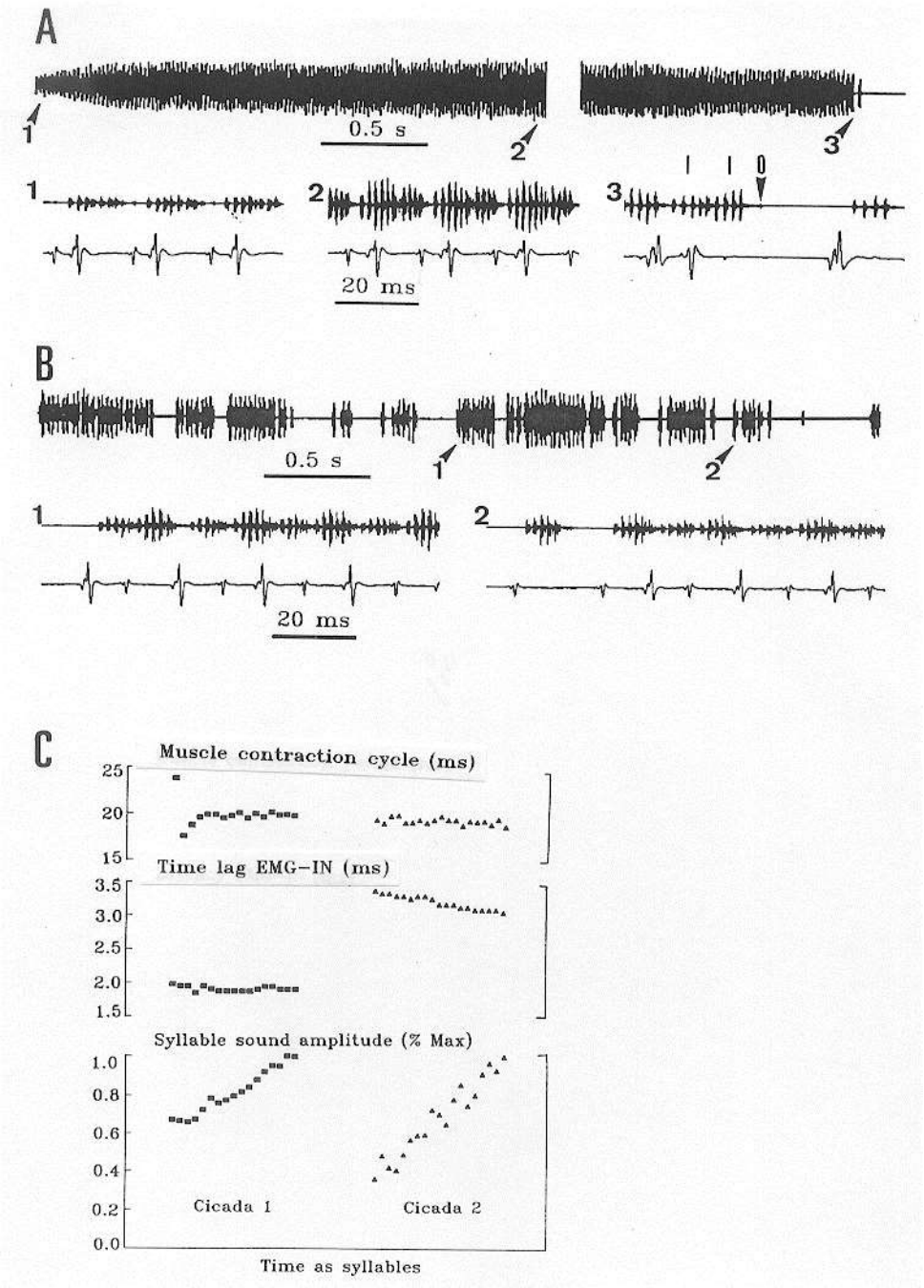


Figure 30 - Sound signals and timbal muscles EMG of *Tibicina quadrisignata*. A) Calling song oscillogram showing (1) the beginning (2) the middle and (3) the end of a sequence. Note the large amplitude changes occurring at the beginning of the sound sequence. B) Oscillogram of an irregular alarm signal. From 1 to 2 expanded sections there is a change on the leading muscle. Both timbal muscle potentials are well represented in the one EMG recorded in one muscle. Note the difference observed in the phase of the bilateral muscles contraction in the calling song (A2) and the alarm signal (B1). I=IN; O=OUT. C) Examples for variation in song parameters (duration of the timbal muscle contraction cycle, time lag from timbal EMG to IN pulse, and syllable sound amplitude) occurring at the beginning of a sequence of the calling song of two males. There is an independence of the sound amplitude relative to the duration of the timbal muscle contraction cycle and to the time lag EMG-IN sound pulse.

The phase of the bilateral muscle contraction was relatively stable, ranging from 0.3-0.4 in the calling song (5 animals). The phase increased to about 0.5 in the alarm signal (4 animals). The time pattern clearly changed from the calling song to the alarm signal (compare EMGs on Fig. 30

A and B), and the overlap of the right-left timbal sounds seen in the calling song was much reduced or absent in the alarm signal. The leading muscle might change in all the signals (calling, courtship and alarm). However these shifts of the leading muscle were much more frequent in the alarm signal (e.g. compare Fig. 30 B1 and B2), where it might change in the middle of the sound sequences. In contrast, in the calling song there were no changes in the leading muscle during a sequence of sound.

The time lag from the timbal muscle EMG to the IN sound pulse was relatively stable during the song, and with about the same values in the calling and alarm signal (values ranged from 1.9 ms to 4.1 ms in 6 animals). However, the time lag was reduced if the animal sang for a while. The reduction in time ranged 21% to 26% in four animals. There was no clear dependence of the IN pulse amplitude on the time lag EMG-IN (Fig. 30 C).

Discussion. 1. The first goal of this investigation was to determine differences and similarities in the timbal muscle activity and the sound pulse pattern between the calling song and the alarm signal. The parameters measured consisted of the amplitude of the sound pulses during single timbal actions, the cycle of the timbal muscles contraction, the intertimbal interval and phase, and the determination of the leading muscle in a song sequence. The data is summarized for all five species in Table 5.

The basic timbal cycle was the same in calling song and alarm signal for all five species, further confirming the data obtained during singing and after inactivating one timbal (chapter 3 b): in *Tett. argentata/atra*, *Tett. josei* and *Tymp. gastrica* there was an IN pulse generated by the inward movement of the timbal and an OUT pulse when the timbal springs back out on elastic force; in *C. barbara* and *C. orni* there were three IN pulses and one OUT sound pulse; in *Tib. quadrisignata* there were up to 7 rib IN pulses and a soft OUT pulse. However, the calling song and alarm signal may be different at several levels. In the calling song there was a definite time and amplitude pattern, sometimes as complex as in *Tett. josei* (Fig. 24 A).

Table 5 - Summary of characteristics of timbal muscles EMGs and their relations with calling songs (C.s.) and alarm signals (A.s.).

Species	*T. argentata/atra*		*T. josei*		*T. gastrica*	*C. barbara lusitanica*		*T. quadrisignata*	
Signal	C.s.	A.s.	C.s.	A.s.	C.s.	C.s.	A.s.	C.s.	A.s.
Time pattern	regular	irregular	regular	irregular	regular	regular	irregular	regular	irregular
Amplitude modulation	regular	variable	regular	variable	regular	regular	no or irregular	regular	no or irregular
N°. Contractions /echeme	3	1-5	2 (1)	1-4	variable	—	—	—	—
Timbal muscles period (ms)	4-8	5.5-10	4.5-13	7.5-8.5	9-18	10-30	9.5-14.5	17-21	18-22
Left/Right interval (ms)	0.7-1.4	0.6-0.8	0.2-0.3	0.4,0.6	2-3	5.5-11	4.5-15	5.5-8	9-11
Timbal muscle phase	0.1	0.1	0.02-0.04	0.05,0.08	0.1-0.2	0.5	0.5	0.3-0.4	0.5
Changes of leading muscle	no	no	no	yes	no	yes	yes	yes	yes
Latency EMG-IN stable in sound sequence	no	variable	no	variable	no	yes	yes	yes	yes
IN-OUT interval (ms)	1.9-4.3	1.9-4.5	2.5-6	3-5	2-8	3.5-4.5	—	—	—
N°. of individuals studied	6	3	6	2	6	3	4	7	5

The alarm signals were always more irregular in time and amplitude. The structure of the echemes might also be different in both signals e.g. in *Tett. argentata/atra* and in *Tett. josei* where the usual 3 and 2 muscle contractions per echeme might change to 4 (1 to 5) and 3 (1 to 4), respectively.

The duration of the cycle of the timbal muscle contractions was variable between cicadas of the same species and even in the same animal, and they depend on the animal's temperature, which is also dependent on the ambient temperature and on previous singing activity. Therefore, the muscle cycle in the calling or in the alarm signals was variable in the same animal with time but it was usually in the same range in the calling song and in the alarm signal. I found longer cycles in the alarm signals more frequently when the animals were not previously calling for some time, and this is in accordance with the observations made by Young and Josephson (1983a). However this picture would usually change if the animals were disturbed immediately after a

period of calling activity. The intertimbal delay was also more regular in the calling song, sometimes with a definite pattern as in *Tib. quadrisignata*.

The phase relationships between the two timbal muscles ranged from near synchrony (0.02-0.04) to full antiphase (0.5) in the species studied (Table 5). Apart from being more stable in the calling song, the phase was similar in both signals, and would usually maintain its constancy even in parts of the calling songs with different duration of the muscles contraction cycles. Only in *Tib. quadrisignata* there was a clear modification of the phase from a stable value in the range 0.3-0.4 in the calling song to full antiphase in the alarm signal. Such phase modifications do not appear to be common in cicadas. Young and Josephson (1983a) report some indirect evidence of a phase shift between calling and alarm signals in two Australian species, Arunta perolata and Tamasa tristigma. The left-right synchrony found in *Tett. josei* is also very unusual in the calling song of cicadas. Only in *Arunta perolata* it was found some indirect evidence of a similar situation (Young and Josephson 1983a).

Changes on the leading muscle were not detected in the calling song of *Tett. argentata/atra*, *Tett. josei* and *Tymp. gastrica*, but they were found on *C. barbara lusitanica* and *Tib. quadrisignata*. However in these cases they were not common and were not detected in the middle of the sound sequences. In contrast, in the alarm signal these changes were detected in all species with alarm signals, possibly with the exception of *Tett. argentata/atra*. In some cases the changes were relatively common and could be found in the middle of the sound sequences. Also common were absences of timbal muscle contractions in the alarm signals, with the same muscle contracting more than once in the sequence. Such suppressions were very uncommon in the calling song.

Thus, cicadas appear to have complex sound producing mechanisms, which may act differently in different species. Even though the basic timbal cycle and the contraction period of the timbal muscles may be the same or very similar in calling song and alarm signal, there are also important differences between the two signals such as the regularity of the pattern and particularly the modifications in the sound pulse amplitude which is often distinct for the calling song, and thus the species. Therefore care must be taken when extrapolating data obtained in the alarm signals to understand the calling song patterns.

2. The second question concerned the characteristic amplitude modulations of the sound pulses seen in the cicada species investigated here. Large changes in the sound pulse amplitude were exhibited by the cicada songs without corresponding changes in the timbal muscle EMG pattern (Figs. 22 A, 24 A, 26 A, 28 A,C and 29 A,C). However, the mechanisms responsible for these changes are apparently not the same for all species.

Evidence for modulation of timbal stiffness: In one group including *Tett. argentata/atra*, *Tett. josei* and *Tymp. gastrica*, there was a positive correlation between the IN sound pulse amplitude and the time lag between the timbal muscle potential and the IN pulse (Figs. 23 A, 25 A, 27 A). Concomitantly a negative relation was found between the time IN-OUT and the time lag EMG-IN (Figs. 23 C, 25 C, 27 C). A likely interpretation of those observations is that the timbal muscle needed a longer time to develop a force enough to overcome the increased timbal stiffness resulting in louder sounds, as suggested by Pringle (1954). The simultaneous reduction in time needed to come back to the resting position also points to a modification in the timbal mechanics (increased convexity and stiffness) and/or faster muscle relaxation, since the elasticity of the timbal allows to bounce back to the outward position when the muscle relaxes. A probable candidate for causing such a modification of the timbal mechanics is the timbal tensor muscle which inserts at the timbal frame. Its action could create the observed amplitude (and latency) changes, as was indicated by the pioneering work of Pringle (1954) and Hagiwara (1955). Indirect but supporting evidence for a tensor muscle involvement comes from the experiments cutting the timbal frame near the insertion sclerite of the tensor muscle in *Tett. argentata/atra* and *Tett. josei*. This lesion most likely disables the tensor action and had the effect that only soft IN pulses occurred during the calling song in those two species.

In *Tett. argentata/atra* the time IN-OUT is apparently affected by two factors: the timbal muscle contraction properties and the modification of the mechanics of the timbal itself which was probably induced by the tensor muscle. The interval IN-OUT usually increased towards the end of the echeme in the alarm signal and this was also observed in the calling song of one animal where the IN sound pulse was soft throughout the echeme. Such an increase was probably related to the contraction properties of the fast timbal muscle, possibly leading to a longer

relaxation time towards the end of the echeme. During the calling song the time IN-OUT either increased in a similar way in the second syllable or stabilized. However, in contrast to the previous situation, it was very much reduced in the third syllable of the echeme (Fig. 23 C) most likely denoting an increase in the stiffness of the timbal membrane that would permit a faster spring out movement.

Evidences of other mechanisms: The other group includes *C. barbara lusitanica*, *C. orni* and *Tib. quadrisignata*. In these cicadas large changes in the amplitude of the sound pulses were not accompanied by changes of the time lag from EMG to IN (Figs. 28 C, 29, 30 C) and there was no correlation between these two measurements. Mechanisms other than the changed stiffness of the timbal are likely to be responsible, possibly at the level of the sound radiation mechanisms that may involve other structures such as abdomen and tympana. The amplitude increase was not correlated with changes in the timbal muscle contraction period in both *C. barbara lusitanica* and *Tib. quadrisignata* (Figs. 28 C, 30 C). A similar situation was found by Aidley (1969) on *Fidicina rana* and by Young and Josephson (1983b) for *Magicicada* species. Even between *Cicada* and *Tibicina* some differences could be seen suggesting that different mechanisms may be involved in creating the changes in sound amplitude. In *Tib. quadrisignata* the amplitude of the sound pulses increased when the animal lifted and extended its abdomen (Fonseca 1991). Thus, the abdominal posture is likely to contribute to the change in sound amplitude. Furthermore, the increase in sound amplitude observed in *Tib. quadrisignata* could be caused by an increase in radiation through the tympana as first suggested by Weber et. al. (1987) to *Magicicada* species and then studied by Young (1990) in *Cyclochila australasiae* and *Macrotristria angularis*, cicadas with the same thick abdominal structure as presented by *Tib. quadrisignata*. In *C. barbara lusitanica* there was no clear evidence for such a role of the abdominal position because large amplitude modulations may occur with very little changes of the abdominal posture. Since in *C. barbara lusitanica* animals with a normal calling song pattern did not produce the large IN pulses in the laboratory, one may think that it was the small window opened in the integument to implant the EMG electrodes that by some reason prevented the production of syllables with the normal amplitude pattern (cf. Fig. 28 A). Although the mechanism responsible for creating the song amplitude modulations in *C. barbara* is unknown, this observation suggests that variations in the

abdomen internal pressure may be involved in the process. Variations in the duration of the timbal muscle contraction cycle: The range in muscle contraction frequencies found in the species studied here is large (50-240 Hz; Table 5) but in accordance with the findings for other cicada species (e.g. Hagiwara 1955; Young and Josephson 1983a,b). The fastest rate (240 Hz) measured in *Tett. argentata/atra* is high for a neurogenic muscle of an insect, but not unique. The fastest rate observed to date was in the cicada *Okanagana vanduzeei* where Josephson and Young (1985) found that the timbal muscle could contract at frequencies of 550 Hz. The katydid *Neoconocephalus robustus* (Josephson and Halverson 1970) and the cicada *Psaltoda claripennis* (Young and Josephson 1983a) might achieve contraction frequencies on their main sound producing muscles of 200 Hz and 224 Hz respectively. In *C. barbara lusitanica*, *Tib. quadrisignata* and *Tymp. gastrica* there was a reduction in the muscle contraction cycle and in the time lag EMG-IN measured with increasing song activity. Since with song activity the muscle temperature rises, this change in temperature may be responsible by the shortening of the duration of the timbal muscle cycle. The reduction in time lag could be caused by a more rapid timbal muscle contraction, which according to Young and Josephson (1983b) is temperature dependent in Magicicada species.

5. Sound modulation

5a. Role of the tensor muscle

Introduction. It was clearly demonstrated above that the sole activity of the timbal muscles was not enough to explain some characteristic variations observed in the cicada songs such as amplitude modulations and time shifts. Therefore research had to consider other elements of the sound producing system that could be responsible for these modifications. The tensor muscle was a possible candidate since it inserts on the timbal frame. Its contraction might modify the mechanics of the timbal and so the sound produced, as suggested by Pringle (1954) and Hagiwara (1955).

The role of the tensor muscle on sound generation was investigated in the same five species of cicadas where the timbal muscles EMG was studied during the generation of the calling song: *Tettigetta argentata/atra*, *Tett. josei*, *Tympanistalna gastrica*, *Cicada barbara lusitanica* and *Tibicina quadrisignata*. First, the activities of the nerve innervating the tensor muscle (tensor nerve) and of the auditory nerve carrying the timbal motoraxon (see page 200) were monitored during singing. A normal calling song pattern was elicited with electrical brain stimulation. Second, the sound produced by the males through electrical stimulation of the auditory nerve (which lead to a contraction of the timbal muscle) or by electrical brain stimulation was recorded during simultaneous electrical stimulation of the tensor nerve. Finally, some effects of the tensor muscle contraction on the mechanics of the timbal were investigated: a) by visual monitoring of the changes induced on the timbal and timbal frame by electrical stimulation of the tensor nerve; b) mimicking the mechanical action of the tensor muscle by pushing on the tensor sclerite during auditory nerve stimulation and singing elicited with electrical brain stimulation; and c) by measuring the force necessary to buckle the timbal inward both with and without tensor nerve stimulation. These experiments allowed a precise study of the effects of the tensor muscle contraction on the sound pulses produced by the timbal buckling.

Results.

Tettigetta argentata/atra. During the calling song echemes, where the timbal muscles contract three times, each of which producing one IN and one OUT sound pulse, the amplitude modulation is characterized by a large progressive increase of the IN pulses, without an observed change in the timbal muscles EMG (Fig. 22 A). From very soft in the beginning, the IN sound pulses become the largest on the third syllable (Fig. 31 A,B). The increase in amplitude of the OUT pulses is less intense.

During the generation of the calling song echemes there was a correlated activity in the tensor nerve (Fig. 31). The pattern of the tensor nerve activity, where several fibers can be recognized, was relatively constant. This activity was similar in both tensor nerves (Fig. 31 B) and was initiated before the generation of the timbal motoneuron spike. It always began with

the firing of the same unit, usually producing only one spike per echeme (Fig. 31 B,C), and was followed by the activity of several other fibers.

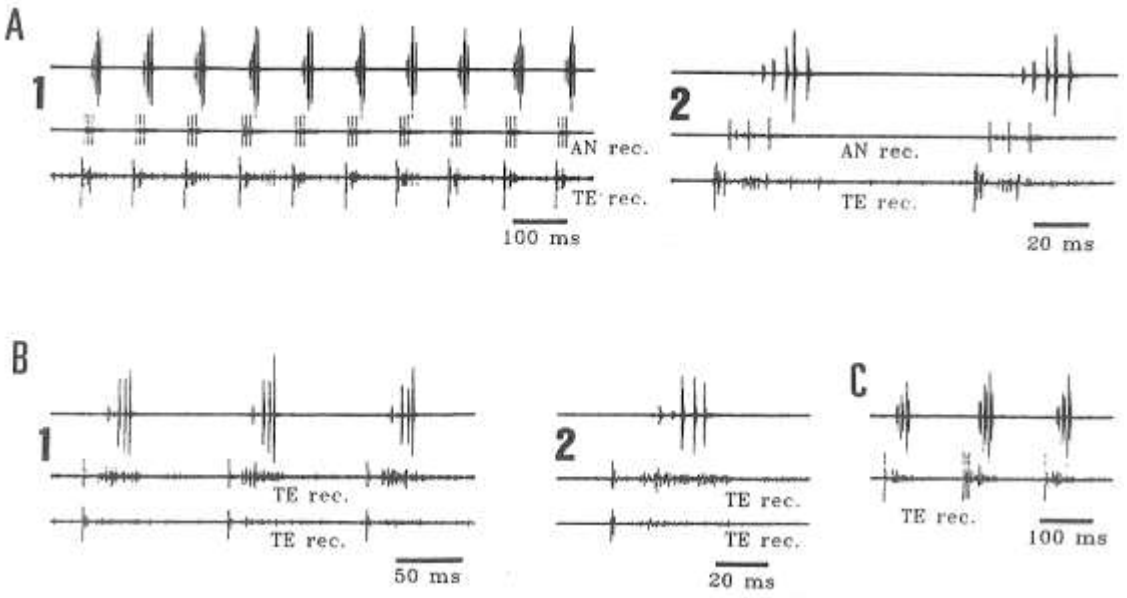


Figure 31 - Auditory nerve and tensor nerve activities during the calling song of *Tettigetta argentata/atra* elicited by electrical brain stimulation. A) Auditory and tensor nerves recording. B) Both tensor nerves recording. C) variation on the tensor nerve activity.

Electrical stimulation of the tensor nerve (100 Hz) during calling song production modified the normal sound amplitude modulation of the echemes (Fig. 32 A,B). Especially the first IN pulses, but also the second ones, became much louder and sometimes they even reached the amplitude of the last IN pulses. A smaller amplitude increase was also seen on the first OUT pulses. Cutting the tensor nerve then abolished the characteristic amplitude modulation (Fig. 32 C). The IN sound pulses remained soft throughout the echeme and the amplitude of the OUT pulses was also reduced.

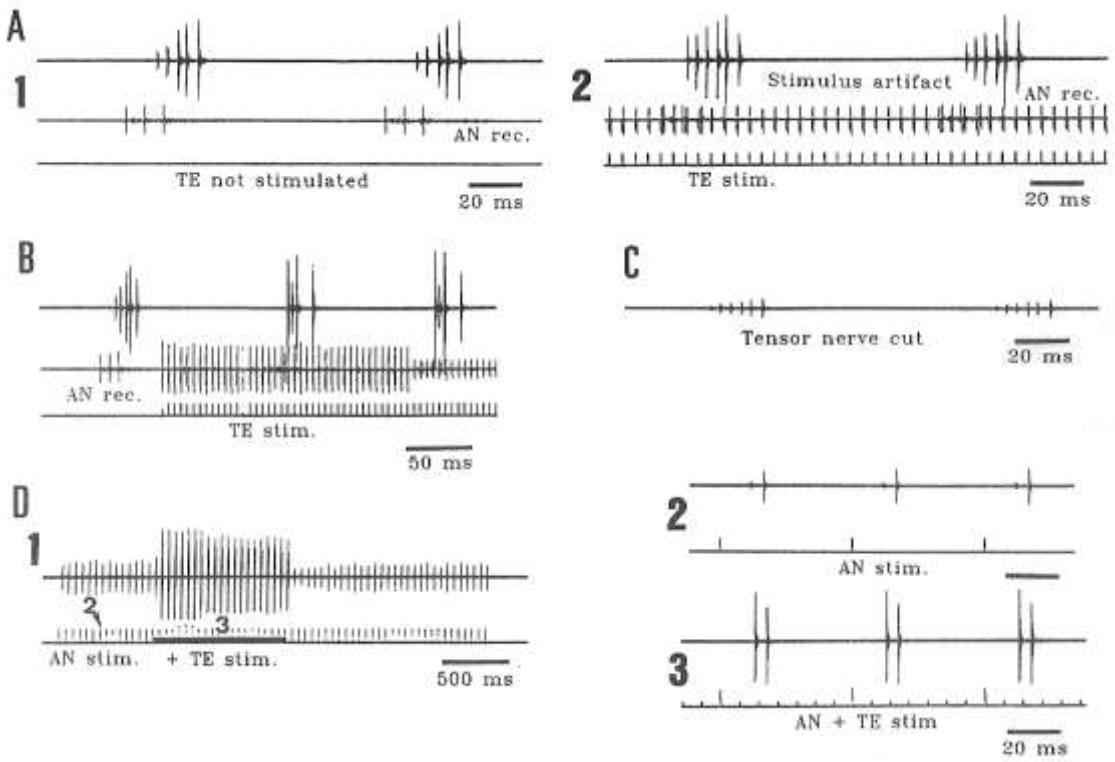


Figure 32 - Effects of electrical stimulation of the tensor nerve in *Tettigetta argentata/atra*, both during one timbal singing elicited by brain stimulation (A, B) and in the sound generated with electrical stimulation of the auditory nerve (D). Notice the large increase of the sound pulses, especially the IN. C) One timbal singing after cutting the tensor nerve; the characteristic amplitude modulation disappears.

Electrical stimulation of the tensor nerve during electrical stimulation of the auditory nerve (Figs. 32 D, 33 A) also resulted in an increase of the amplitude of the IN and OUT pulses. The amplitude reached by both the IN and the OUT sound pulses was dependent on the frequency of the tensor nerve stimulation (Fig. 34 A) as that should result in an increased contraction strength of the tensor muscle with increasing stimulation frequency. The amplitude increased through 20 Hz to 80 Hz of tensor nerve stimulation rates and then stabilized in loud sound pulses. Concomitantly there was an increase in the latency measured from the electrical stimulus of the auditory nerve and the sound pulse generated (Fig. 34 B). This increase would reach about 1-1.5 ms on average. The amplitude of the sound pulses, especially the IN, was positively correlated with the latency auditory nerve stimulus-to-sound (Fig. 35). The time interval between IN and OUT pulses was also reduced by the electrical stimulation of the tensor nerve (cf. Figs. 32, 33 B). However, the effects of an increased rate of tensor nerve stimulation may not be the only determinant to the observed enhancement on amplitude and latency since the effectiveness of

the tensor nerve stimulation was also dependent on its timing relative to the auditory nerve stimulation.

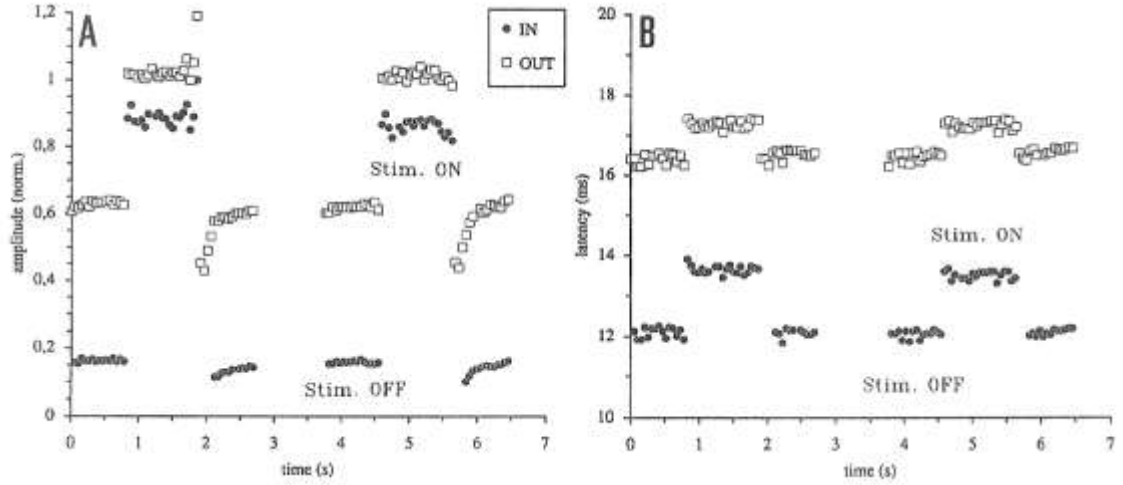


Figure 33 - Variations on the IN and OUT sound pulses amplitude (A) and on the latency from the auditory nerve stimulus to sound (B), induced over time by electrical stimulation of the tensor nerve at a rate of 200 Hz during electrical stimulation of the auditory nerve at 20 Hz, on one male of *Tettigetta argentata/atra*.

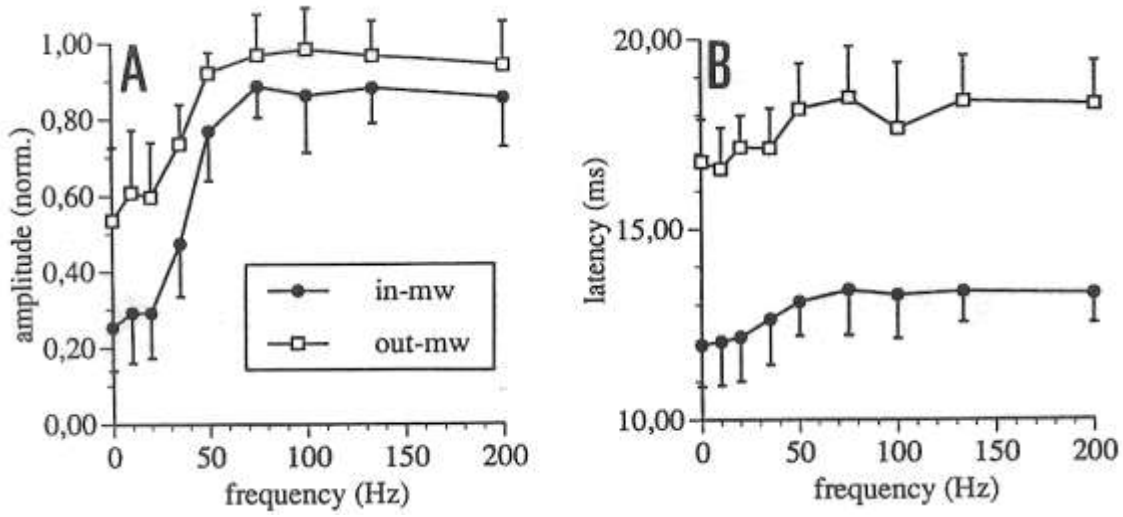


Figure 34 - Averaged effects on the IN and OUT sound amplitude (A) and on the latency from auditory nerve stimulus (20 Hz) to sound (B), caused by increasing frequencies of tensor nerve stimulation on 5 individuals of *Tettigetta argentata/atra*. Error bars correspond to standard deviations of the weighed means.

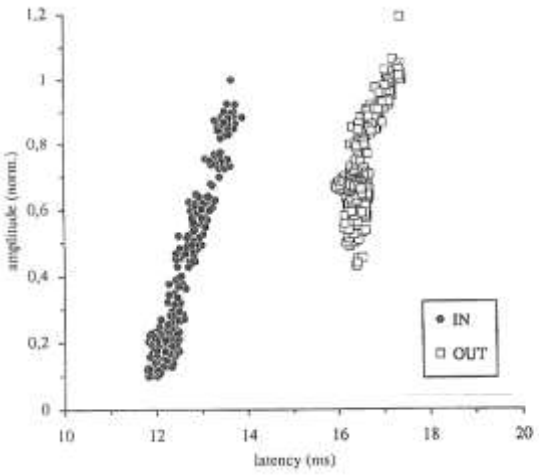


Figure 35 - Dependence of the sound pulses amplitude on the relative timing between tensor nerve and auditory nerve electrical stimulations in one male of Tettigetta argentata/atra (two series). There is a time window within which one tensor stimulus can elicit a large increase of the IN pulse amplitude.

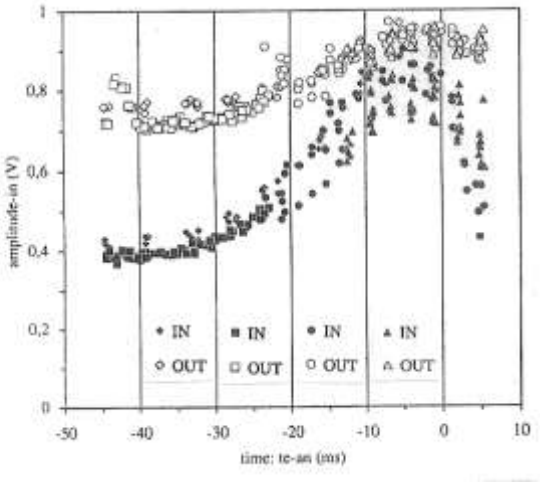


Figure 36 - Relationships between the IN and OUT sound pulses amplitude and the latency from auditory nerve stimulus to sound, during the experiments with auditory and tensor nerves electrical stimulation in one male of Tettigetta argentata/atra. The clear positive correlation found in the IN pulse is less definite in the OUT.

The effect on the amplitude of the sound pulse generated was maximal if the tensor nerve stimulus came within a time window ranging from 10 ms before it occurred synchronous with the auditory nerve stimulus (Fig. 36). One stimulus produced within this time window was in

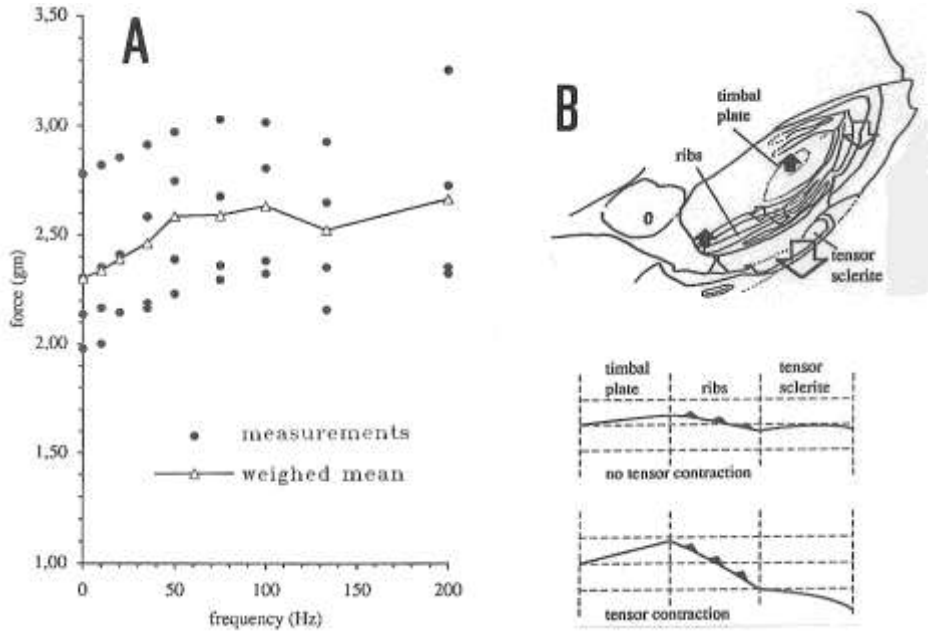


Figure 37 - A) Averaged effects of the rate of electrical stimulation of the tensor nerve on the spring force needed to buckle the timbal inward in *Tettigetta argentata/atra* (2 measures on 2 individuals). B) Modifications of the timbal and timbal frame induced by effective tensor nerve stimulation showed on a perspectively flattened view of the timbal and by a schematic crossection perpendicular to the ribs. The arrows indicate areas of the timbal moving inward (white arrows) and outward (black arrows) due to contraction of the tensor muscle.

some preparations sufficient to provoke a large effect (80-90%).

Visual observation showed that electrical stimulation of the tensor nerve modified the profile of the timbal and timbal frame (Fig. 37 B), essentially increasing its convexity, and therefore modifying its mechanics. The force necessary to buckle the timbal inward was gradually intensified with increasing rates of tensor nerve stimulation. At high frequencies of stimulation this increment was 0.35 gm on average (Fig. 37 A).

Tettigetta josei. During the echemes on Part I of the complex phrase of the calling song the timbal muscles contract two times almost in synchrony, each of which producing one IN and one OUT sound pulse (cf. Fig. 17). While the first IN pulses of each echeme on Part I are very soft, the second IN pulses are the loudest, much noisier than the OUT pulses that remain similar throughout the echeme (Fig. 38 A). This characteristic pattern of amplitude modulation is not correlated with modifications on the timbal muscles EMG (Fig. 24 A).

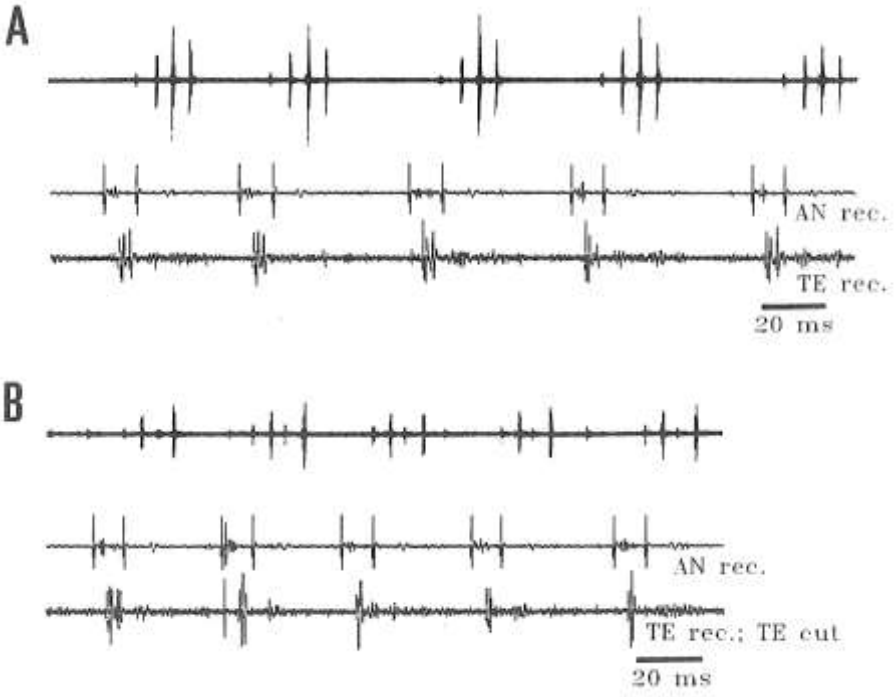


Figure 38 - Auditory nerve and tensor nerve activities during the calling song of *Tettigetta josei* elicited by electrical brain stimulation. A) Auditory and tensor nerves recording. B) The same recordings after cutting the tensor nerve; the IN sound pulses become similar throughout the echemes.

During the generation of the calling song echemes there was an activity on the tensor nerve tightly correlated with the increase of the sound pulse amplitude (Fig. 38 A). This spiking activity occurred between the two spikes of the timbal motoneuron characteristic of one echeme.

Electrical stimulation of the tensor nerve during singing elicited with brain stimulation modified the characteristic sound amplitude modulation, and the first IN pulses of each echeme became as loud as the second ones (Fig. 39 A). There was only a very small effect on the OUT pulses. With the tensor nerves cut during sound production by the male the IN pulses became very soft throughout the echeme (Fig. 38 B).

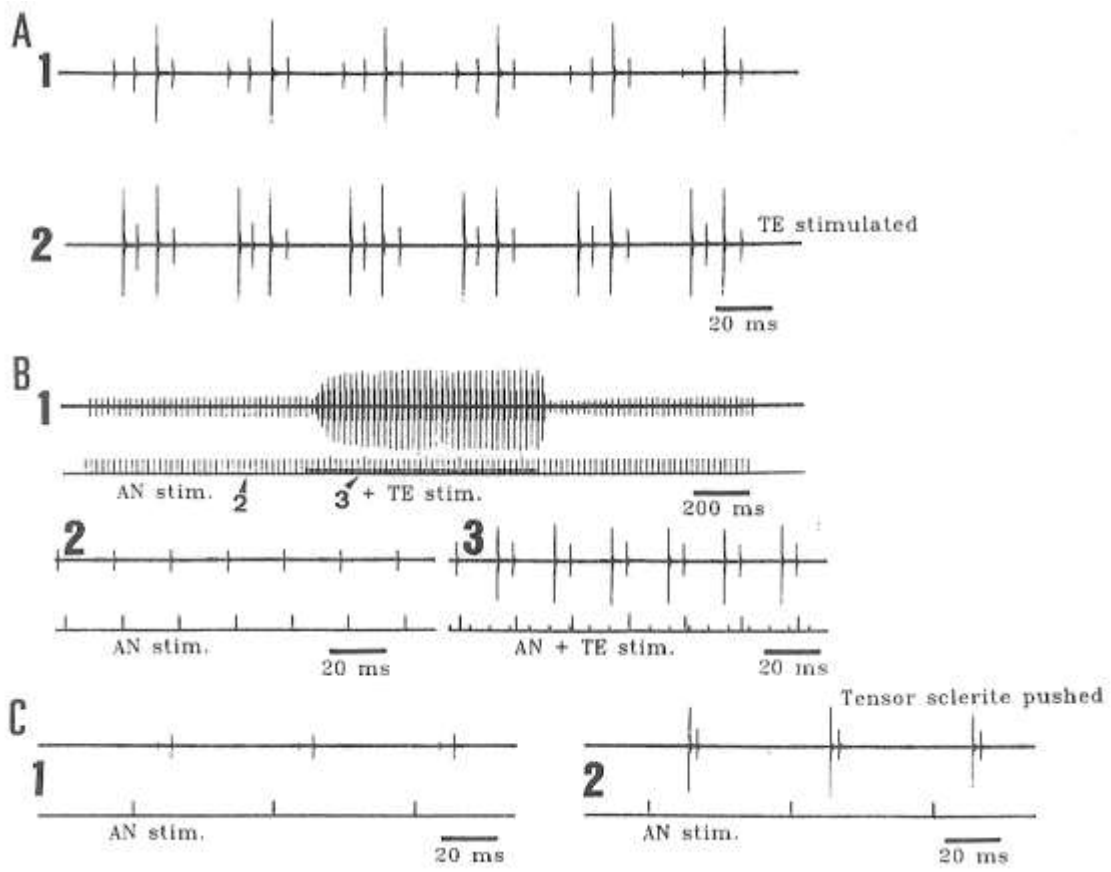


Figure 39 - Effects of electrical stimulation of the tensor nerve in *Tettigetta josei*, both during one timbal singing elicited by brain stimulation (A) and in the sound generated with electrical stimulation of the auditory nerve (B). Notice the large increase of the IN pulses. C) The same effect could be obtained by mechanically pushing the sclerite where the tensor muscle attaches on the timbal frame, during electrical stimulation of the auditory nerve.

A similar increase on the sound amplitude and time shifts could be induced with electrical stimulation of the tensor nerve during the stimulation of the auditory nerve (Figs. 39 B, 40 A). It shall be noted that in this species the full effect on the amplitude and latency shifts only developed after some time upon turning on the tensor stimulation (cf. Figs. 39 B, 40). A mechanical push on the sclerite where the tensor muscle attaches on the timbal frame was enough to provoke an identical change in amplitude and latency as during electrical stimulation of the tensor nerve (Fig. 39 C).

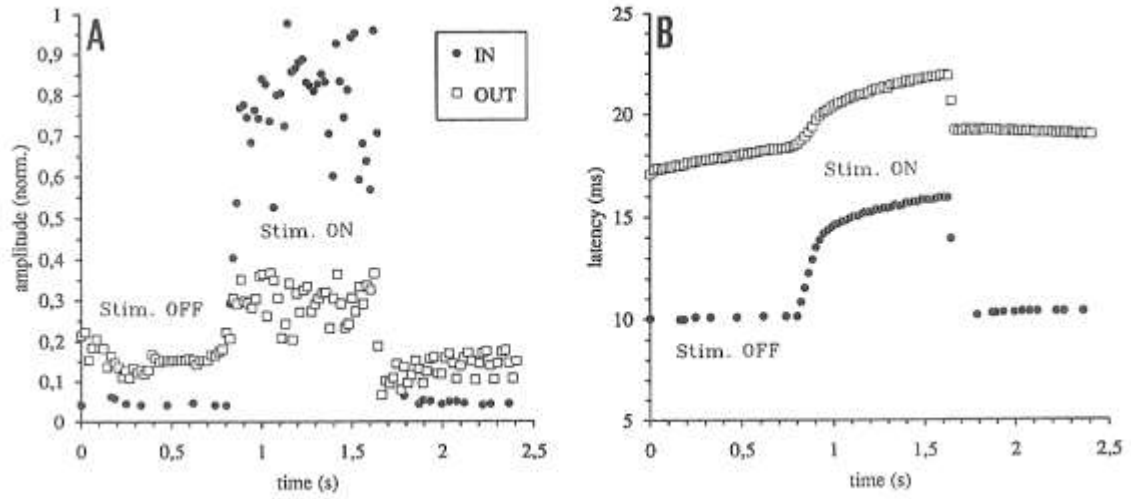


Figure 40 - Variations on the IN and OUT sound pulses amplitude (A) and on the latency from the auditory nerve stimulus to sound (B), induced over time by electrical stimulation of the tensor nerve at a rate of 200 Hz during electrical stimulation of the auditory nerve at 20 Hz, on one male of *Tettigetta josei*. Some time is needed to obtain full effects after the onset of the stimulation.

The amplitude of the sound pulses, especially the IN, was dependent on the frequency of the tensor stimulation (Fig. 41 A). The amplitude had an accentuated progressive increase from 30 to 100 Hz of tensor nerve stimulation rates, and the IN pulses

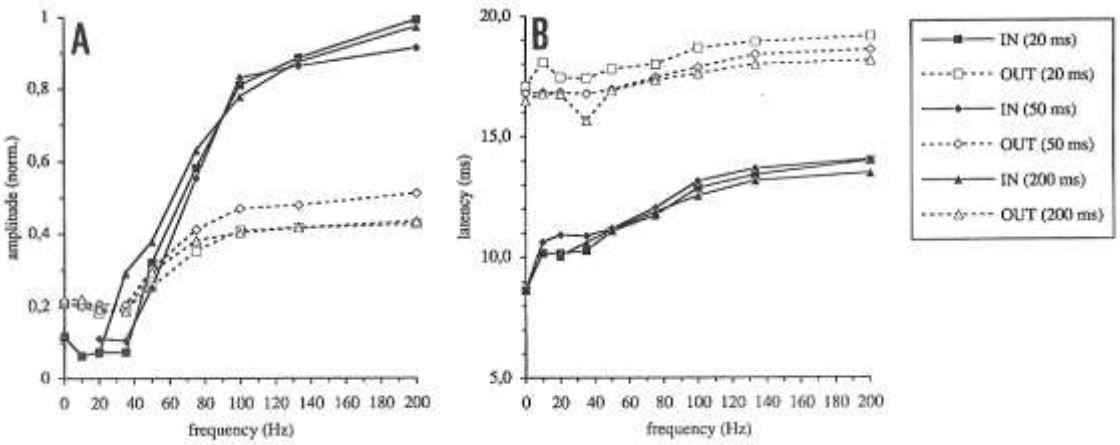


Figure 41 - Averaged effects on the IN and OUT sound amplitude (A) and on the latency from auditory nerve stimulus (20, 50 and 200 Hz) to sound (B), caused by increasing frequencies of tensor nerve stimulation on 5 individuals of *Tettigetta josei*.

would still slightly increase with higher rates of stimulation up to 200 Hz. Concomitantly there was also an increase in the latency from the electrical stimulus of the auditory nerve to the sound pulse generated, reaching more than 3 ms on average (Fig. 41 B). The amplitude of the IN sound pulses was positively correlated with the latency measured from the auditory nerve stimulus to

the sound pulse (Fig. 42). Such correlation did not seem to occur on the OUT pulses. The time interval between IN and OUT sound pulses was also reduced by the electrical stimulation of the tensor nerve (cf. Figs. 39 B2,3 and 40 B).

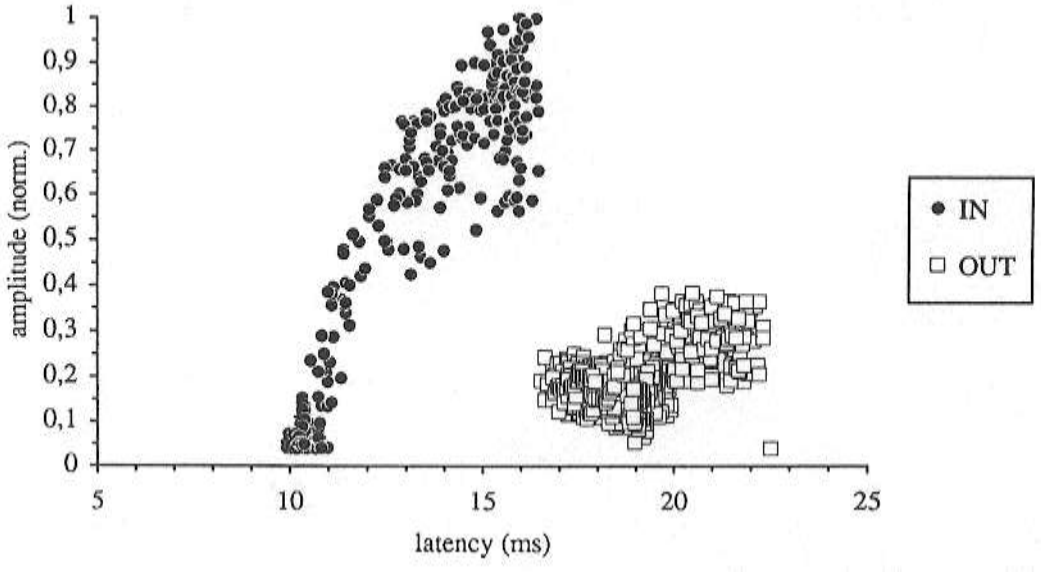


Figure 42 - Dependence of the sound pulses amplitude on the relative timing between tensor nerve and auditory nerve electrical stimulations in one male of *Tettigetta josei* (two series). There is a time window within which one tensor stimulus can elicit a large increase of the IN pulse amplitude.

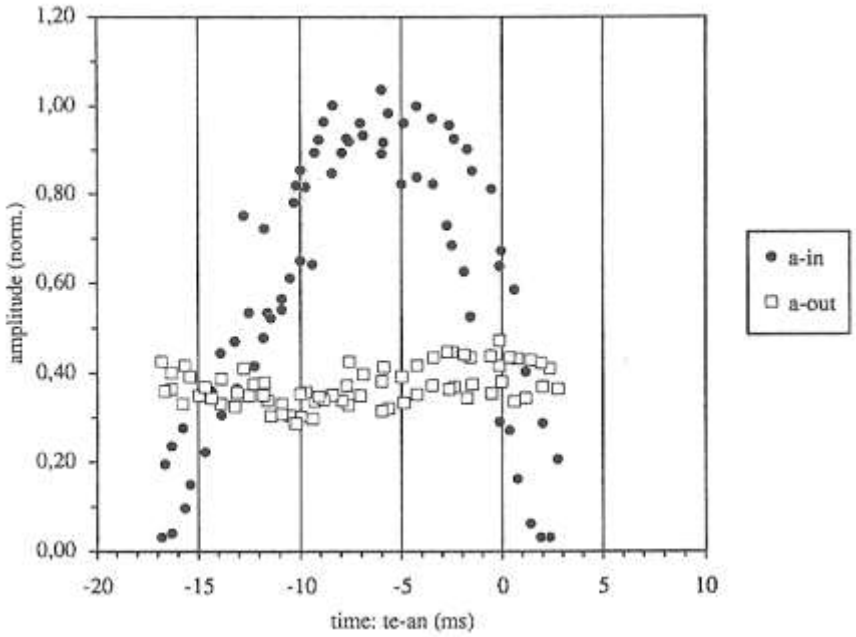


Figure 43 - Relationships between the IN and OUT sound pulses amplitude and the latency from auditory nerve stimulus to sound, during the experiments with auditory and tensor nerves electrical stimulation in one male of Tettigetta josei. A clear positive correlation was found in the IN pulse but not in the OUT pulse.

However, as in *Tett. argentata/atra*, one electrical stimulus delivered to the tensor nerve with a correct timing could elicit large modulations. Here the effect on the amplitude of the IN sound

pulse generated by a single stimulus pulse was maximal if the tensor nerve stimulus came within about 10-3 ms before the auditory nerve stimulus (Fig. 43).

Visual observation showed that the electrical stimulation of the tensor nerve modified the shape of the timbal and timbal frame (Fig. 44 B) resulting, as in *Tett. argentata/atra*, in an increased convexity of the timbal and thus in a change of its mechanics. The force necessary to buckle the timbal inward gradually increased with increasing rates of tensor nerve stimulation up to 80 Hz. The maximal increment of this force was 0.4 gm on average (Fig. 44 A).

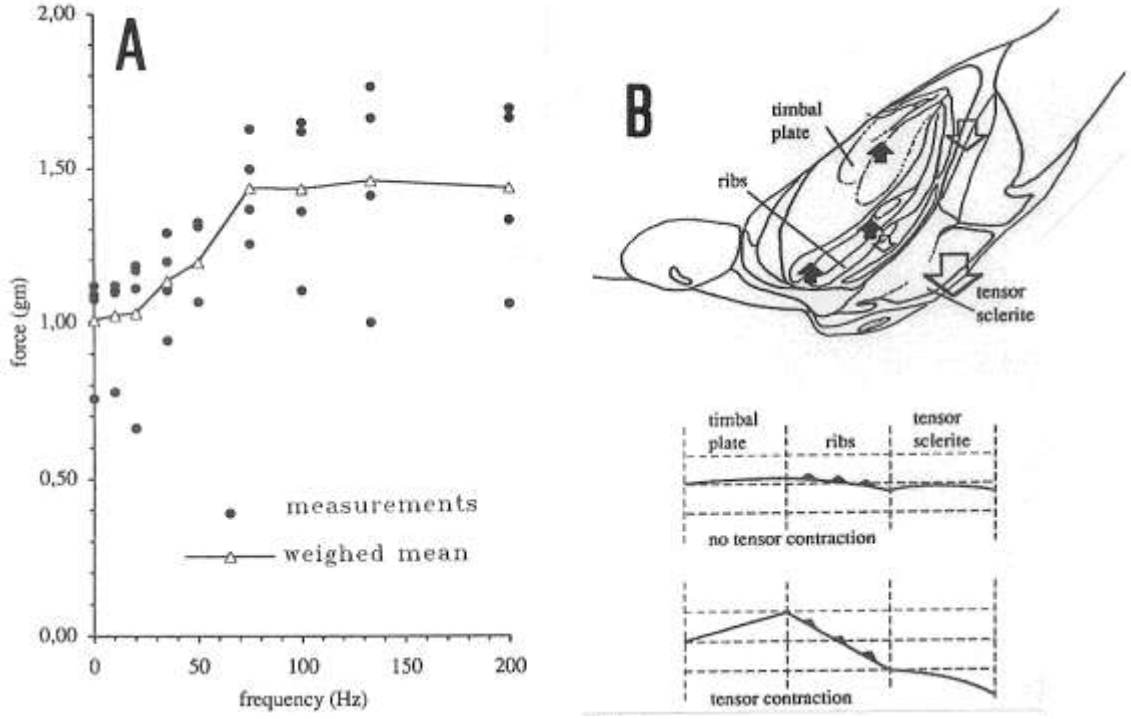


Figure 44 - A) Averaged effects of the rate of electrical stimulation of the tensor nerve on the spring force needed to buckle the timbal inward in *Tettigetta josei* (2 measures on 2 individuals). B) Modifications of the timbal and timbal frame induced by effective tensor nerve stimulation showed on a perspectively flattened view of the timbal and by a schematic crossection perpendicular to the ribs. The arrows indicate areas of the timbal moving inward (white arrows) and outward (black arrows) due to contraction of the tensor muscle.

Tympanistalna gastrica. The calling song echemes begin with loud IN pulses, which then become much softer and similar to the OUT pulses (Fig. 45). The change of the pulse amplitude occurs without modifications in the timbal muscles EMG (cf. Fig. 26 A). Each timbal cycle generates one IN and one OUT sound pulse.

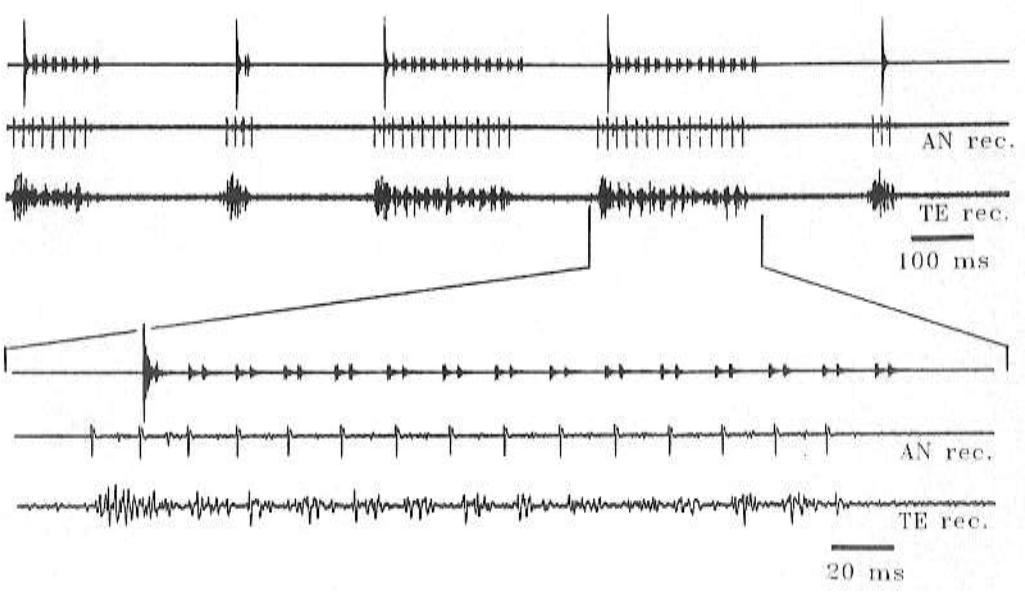


Figure 45 - Auditory nerve and tensor nerve activities during the calling song of Tympanistalna gastrica elicited by electrical brain stimulation.

During the generation of the calling song echemes there was a correlated activity on the tensor nerve linked in time with a decrease in the sound pulse amplitude (Fig. 45). The discharges in the tensor nerve started soon after the first spike of the timbal motoneuron and lasted during the whole echeme. This activity was larger at the beginning and then remained constant and related to each next timbal motoneuron spike.

Lesioning the tensor nerves during calling song production modified the characteristic pattern of the amplitude modulation and time shifts of the sound pulses on the calling song echemes (Fig. 46) induced with brain stimulation. The timbal ipsilateral to the cut tensor nerve produced only loud IN pulses, while the contralateral timbal could still generate the normal pattern (Fig. 46 B). The lesion of the second tensor nerve prevented the generation of soft IN sound pulses entirely (Fig. 46 C).

A similar decrease of the amplitude and time shifts of the IN sound pulse could be obtained either by tensor nerve stimulation (Fig. 47 A) or by pushing on the sclerite where the tensor muscle inserts at the timbal frame (Fig. 47 B), both during electrical stimulation of

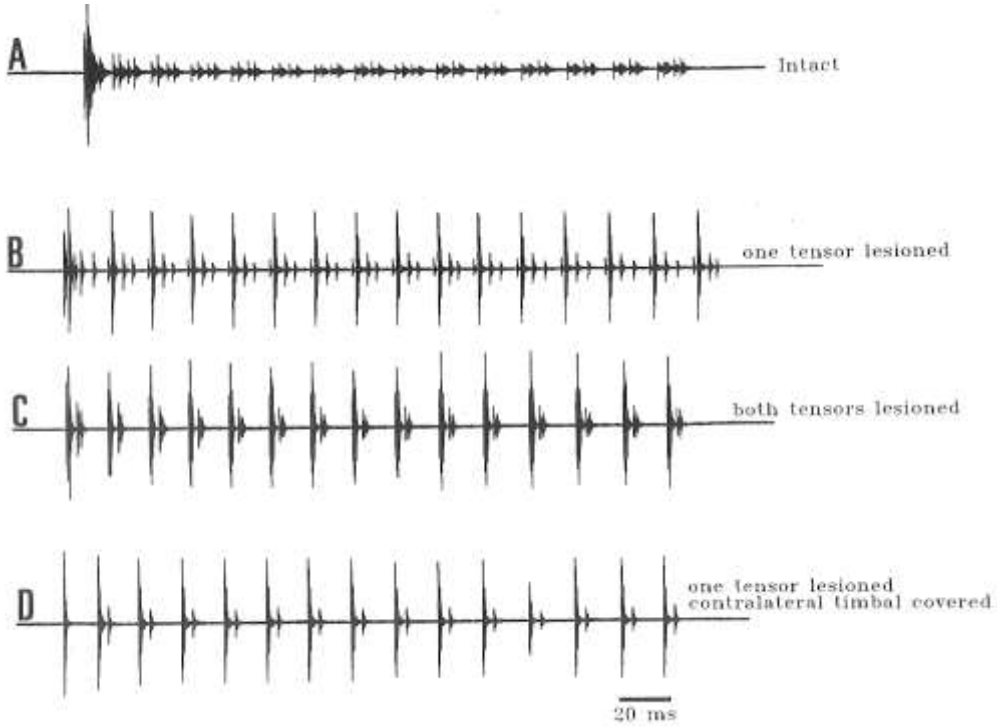


Figure 46 - Effects of lesioning the tensor nerve on the calling song of one male of *Tympanistalna gastrica* elicited by electrical brain stimulation. A) Calling song echeme with the tensor nerves intact. B) One tensor nerve lesioned. C) Both tensor nerves lesioned. D) Tensors lesioned and one timbal covered with vaseline, preventing it to generate sound. The IN sound pulses become large throughout the echeme after lesioning the correspondent tensor nerve.

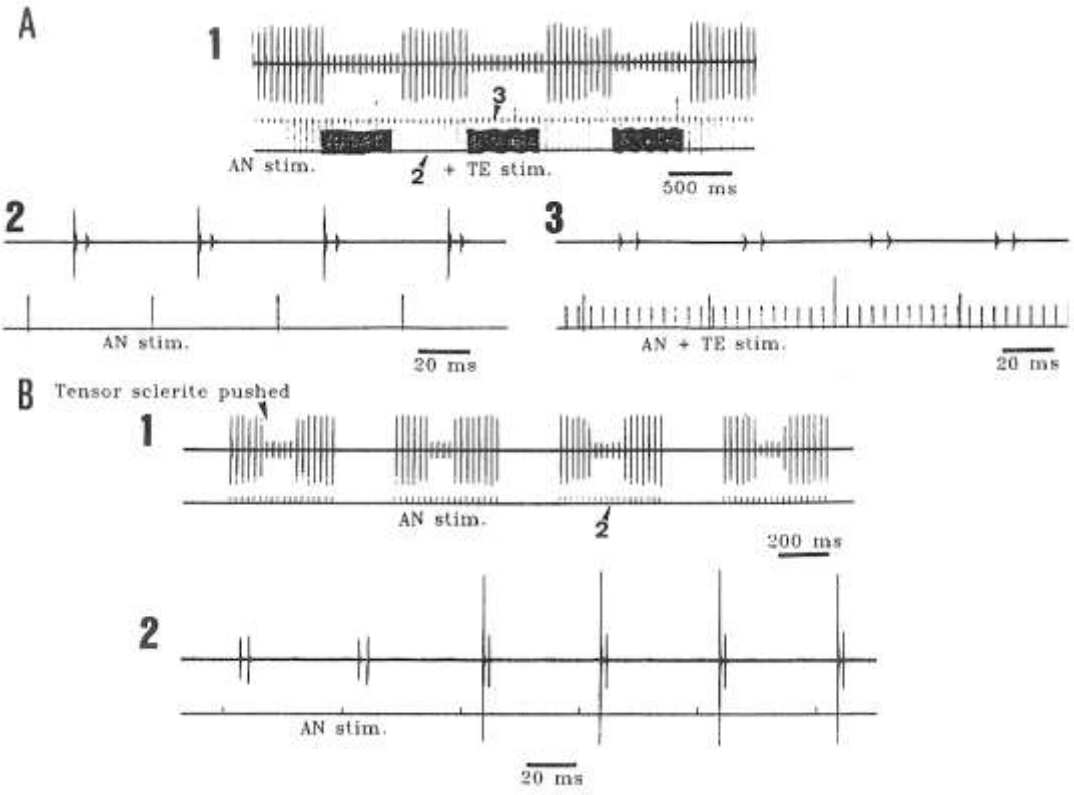


Figure 47 - A) Effects of electrical stimulation of the tensor nerve in *Tympanistalna gastrica* on the sound induced with electrical stimulation of the auditory nerve. Notice the large decrease of the IN pulses. B) The same effect could be obtained by mechanically pushing the sclerite where the tensor muscle attaches on the timbal frame, during electrical stimulation of the auditory nerve.

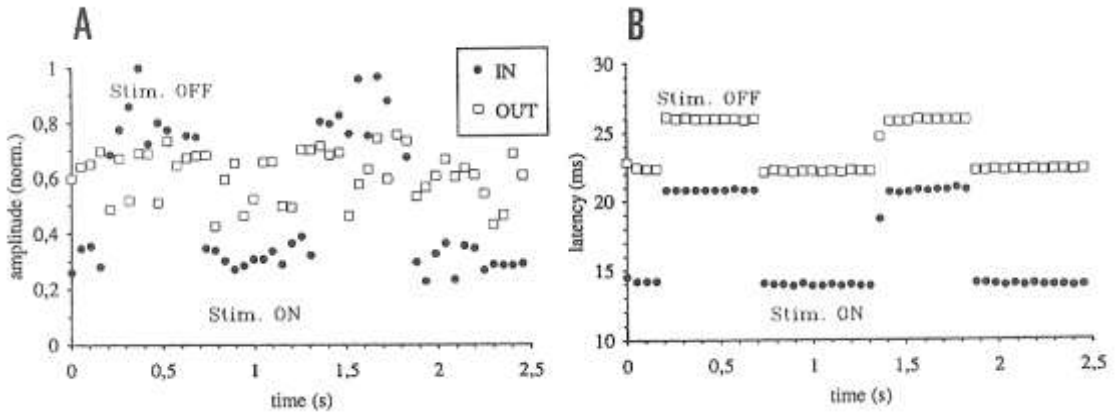


Figure 48 - Variations on the IN and OUT sound pulses amplitude (A) and on the latency from the auditory nerve stimulus to sound (B), induced over time by electrical stimulation of the tensor nerve at a rate of 200 Hz during electrical stimulation of the auditory nerve at 20 Hz, on one male of *Tympanistalna gastrica*.

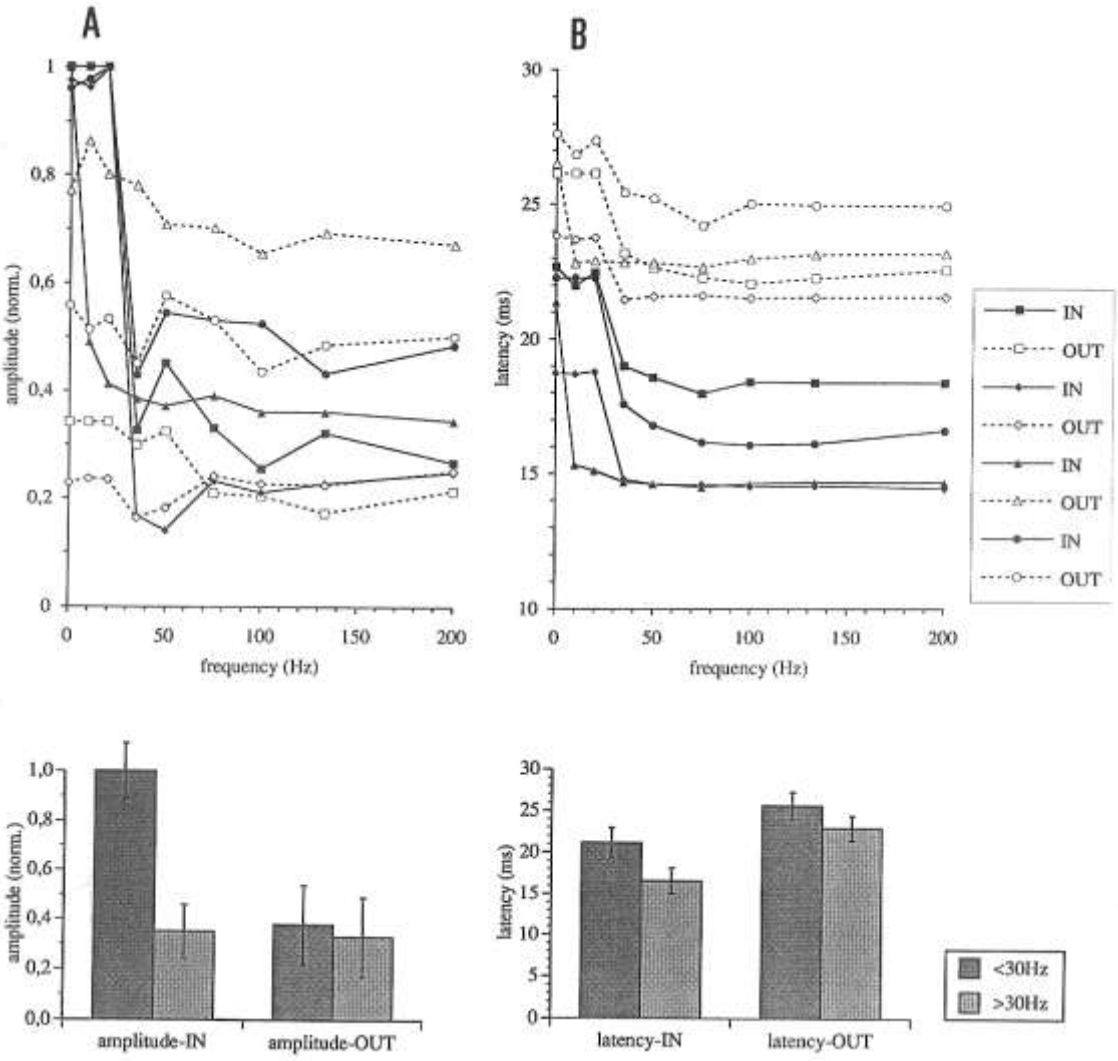


Figure 49 - Averaged effects on the IN and OUT sound amplitude (A) and on the latency from auditory nerve stimulus (20 Hz) to sound (B), caused by increasing frequencies of tensor nerve stimulation on 4 individuals of *Tympanistalna gastrica*. The effects shifted around 30 Hz of tensor stimulation and the differences are significative for the IN but not for the OUT pulses (> 95% confidence).

the auditory nerve. However, the IN pulse would become soft suddenly while increasing the rate of tensor nerve stimulation (Fig. 49 A) or moving the sclerite inward progressively (Fig. 47 B). The amplitude of the IN sound pulses as well as the latency measured from the auditory nerve stimulus to the sound pulse would abruptly decrease around 30 Hz of tensor nerve stimulation (Fig. 49). The decrease in the latency to the IN pulse was about 4 ms on average (Figs. 48 B, 49 B) while the amplitude of the IN pulse showed a 3 fold reduction (Figs. 48 A, 49 A). The OUT sound pulse did not change much in amplitude during the electrical stimulation of the tensor nerve (Figs. 48 A, 49 A). The time interval between IN and OUT sound pulses was suddenly increased while increasing the tensor nerve stimulation rate (cf. Fig. 47 A2,3).

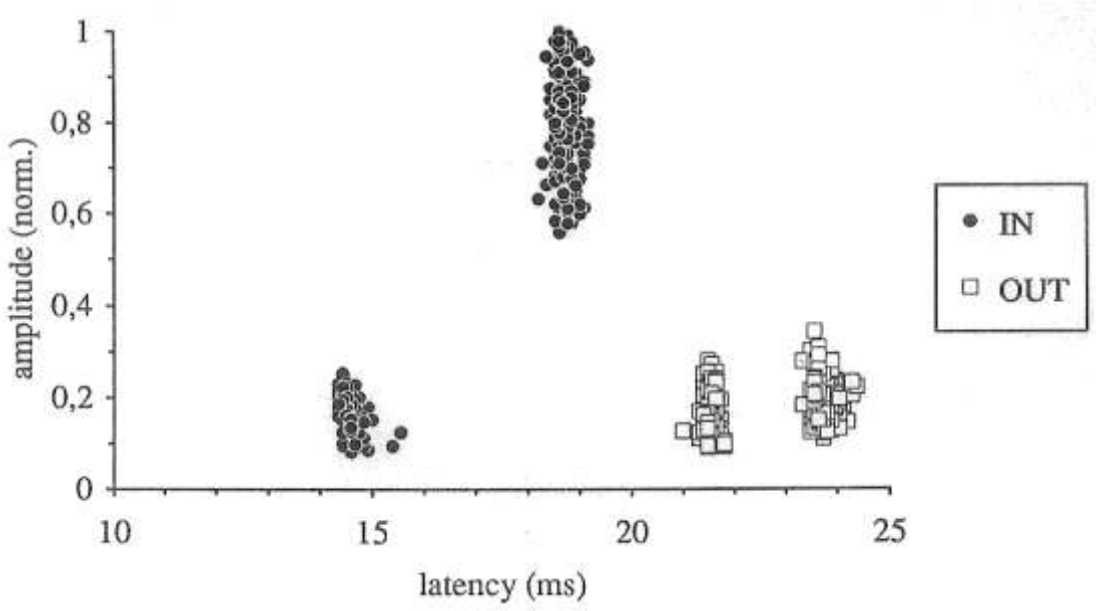


Figure 50 - Dependence of the sound pulses amplitude (A) and of the latency from the auditory nerve stimulus to sound (B) on the relative timing between tensor nerve and auditory nerve electrical stimulations in one male of *Tympanistalna gastrica* (two series). There is a time window within which one tensor stimulus can elicit a large decrease of the IN pulse amplitude and latency.

The amplitude of the IN sound pulses was positively correlated with the latency from the auditory nerve stimulus to the sound (Fig. 50). Such a correlation did not occur with the OUT pulses. However, the above mentioned dependence of the pulse amplitudes and latencies on the tensor nerve stimulation rate may not be the only determinant since, to become effective, the electrical stimulus of the tensor nerve had to occur within a time window extending from about 40 to 5 ms before the auditory nerve stimulus (Fig. 51 A,B). Moreover, a single tensor nerve stimulus seemed to be effective if delivered within this time window.

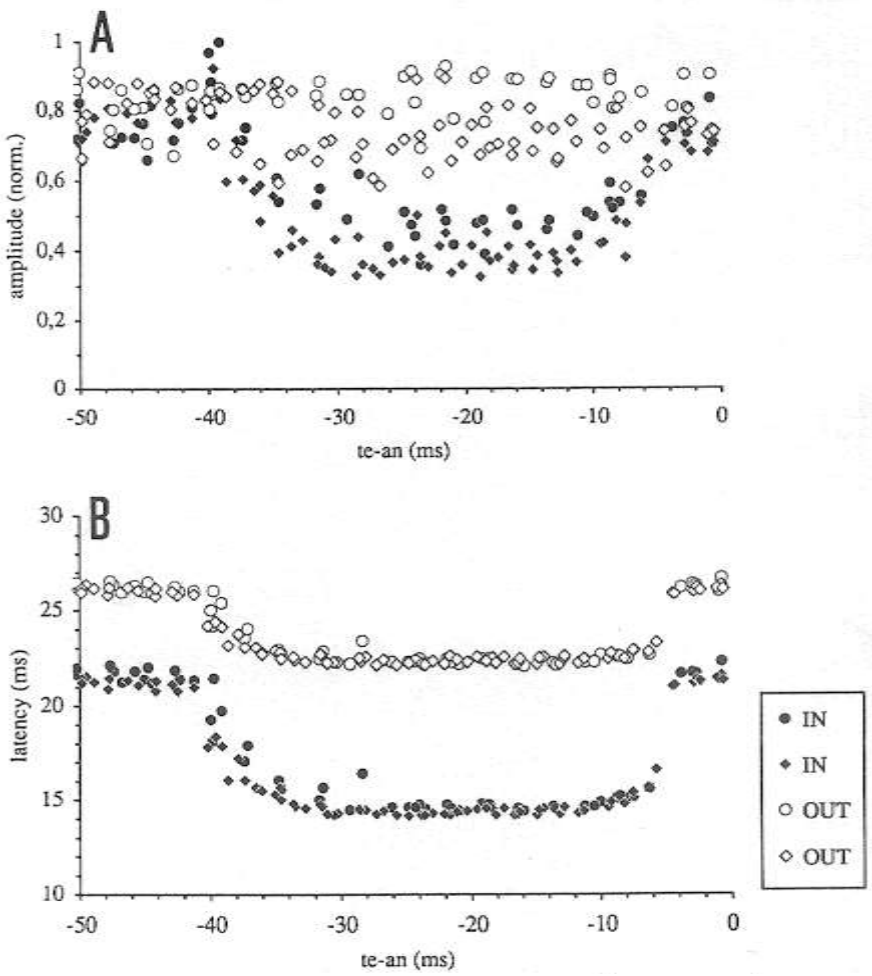


Figure 51 - Relationships between the IN and OUT sound pulses amplitude and the latency from auditory nerve stimulus to sound, during the experiments with auditory and tensor nerves electrical stimulation in one male of *Tympanistalna gastrica*. A clear positive correlation was found in the IN pulse but not in the OUT pulse.

Visual observation showed that the electrical stimulation of the tensor nerve modified the shape of the timbal and timbal frame (Fig. 52 B), however, in *Tymp. gastrica* decreasing its convexity and so modifying its mechanics. The force needed to buckle the timbal inward was reduced for stimulation frequencies of the tensor nerve above 30 Hz. This reduction was relatively small, averaging 0.07 gm (Fig. 52 A).

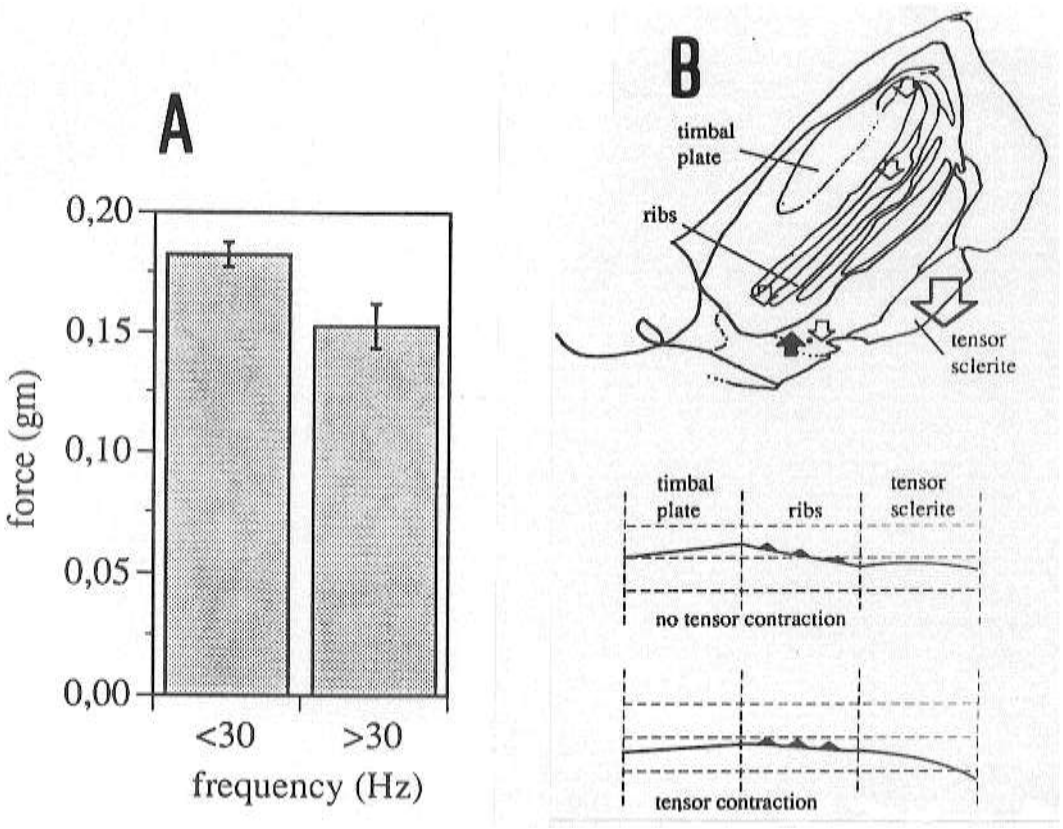


Figure 52 - A) Averaged effects of rates of electrical stimulation of the tensor nerve below and above 30 Hz on the spring force needed to buckle the timbal inward in *Tympanistalna gastrica* (mean values and standard deviations; from 2 males). The force decreased abruptly at stimulation rates of the tensor nerve above 30 Hz. B) Modifications of the timbal and timbal frame induced by effective tensor nerve stimulation showed on a perspectively flattened view of the timbal and by a schematic crossection perpendicular to the ribs. The arrows indicate areas of the timbal moving inward (white arrows) and outward (black arrows) due to contraction of the tensor muscle. There was also a joint like movement in the timbal frame ventral to the tensor sclerite indicated by a dot and two oppositely directed arrows.

Cicada barbara lusitanica. In the calling song of this species there is an increase of the sound amplitude at the beginning of a calling song sequence (cf. Fig. 9 A), and the IN sound pulses increase strongly becoming louder than the OUT pulse (Fig. 9 A2,3).

The brain stimulation failed to evoke the normal syllable pattern of amplitude modulation, although a continuous sound sequence was easily obtained as an after effect of the stimulation (Figs. 53 A, 54 A1).

During sound generation there was increased activity on the tensor nerve (Fig. 53 A). However, the strict correlation with the timbal motoneuron activity in the auditory nerve recording as found for the previous species was not seen (cf. Figs. 31, 38, 45). There was increased activity in the thick medial abdominal nerves correlated with sound production as well (Fig. 53 B).

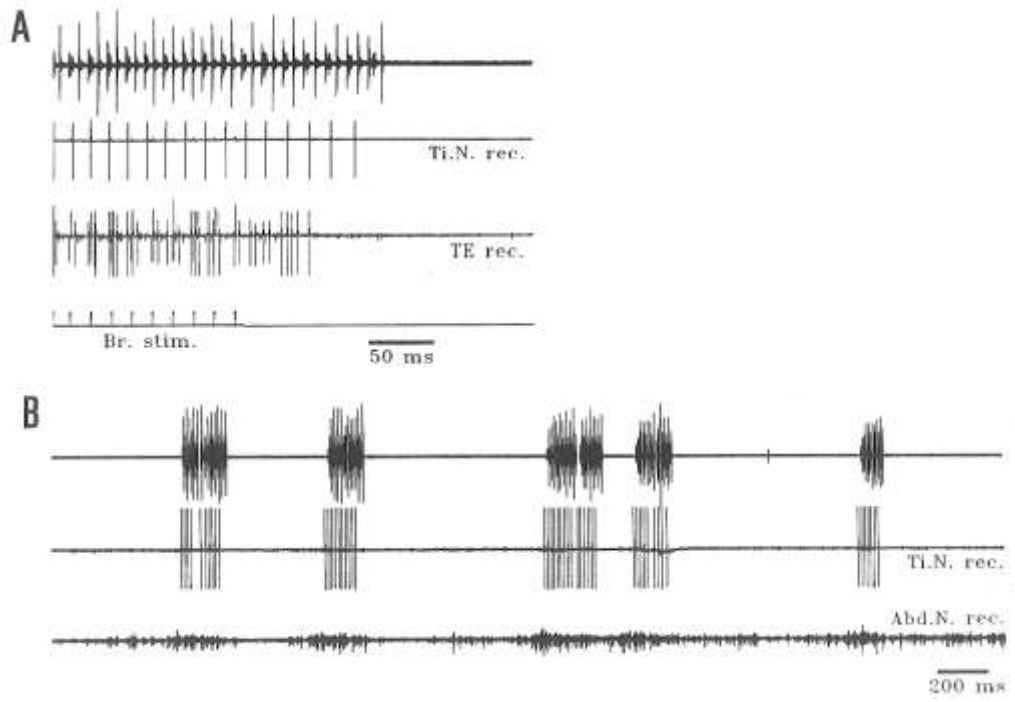


Figure 53 - Timbal nerve activity and A) tensor nerve or B) medial large abdominal nerve activities during singing elicited by electrical brain stimulation on *Cicada barbara lusitanica*.

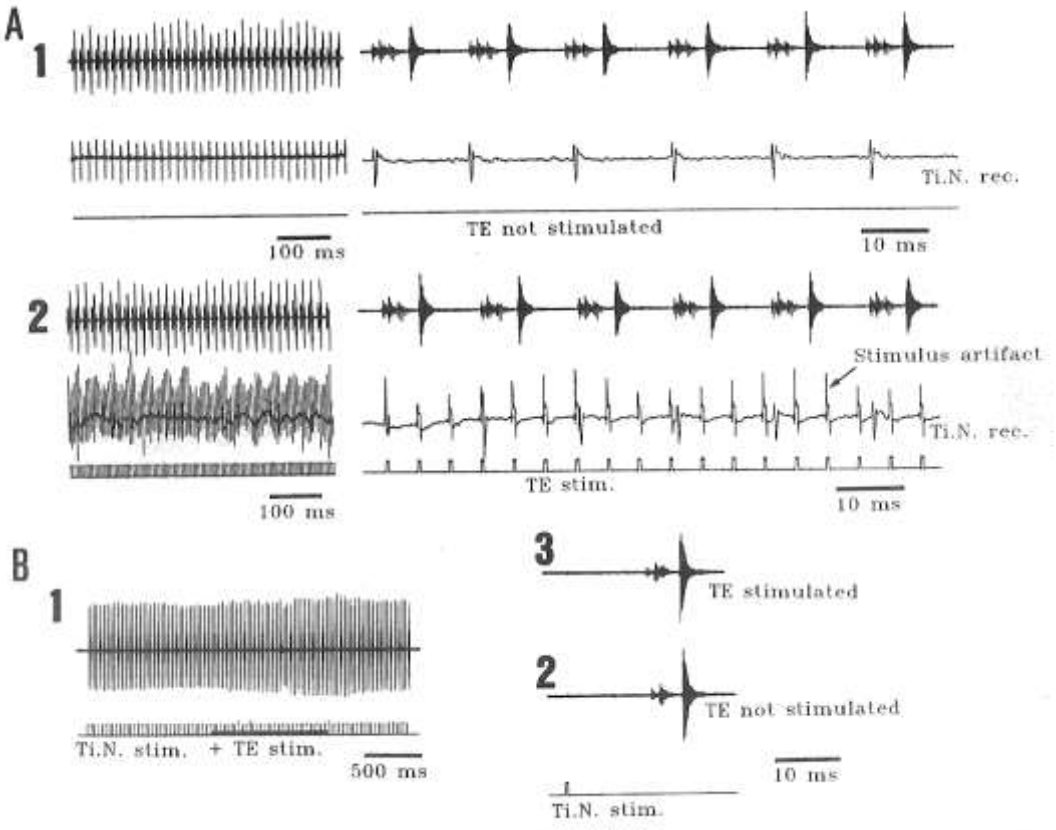


Figure 54 - Effects of electrical stimulation of the tensor nerve in *Cicada barbara lusitanica*, both during one timbal singing elicited by brain stimulation (A) and in the sound generated with electrical stimulation of the timbal nerve (B). The effects in amplitude were usually small or inexistent; although, sometimes the 3rd IN pulse could arise during tensor stimulation (B2,3).

Electrical stimulation of the tensor nerve did not produce large effects neither during singing obtained by brain stimulation (Fig. 54 A) nor during electrical stimulation of the timbal motoraxons (Fig. 54 B). However, sometimes the third IN pulse appeared, apparently induced by the electrical stimulation of the tensor nerve (Fig. 54 B2,3). Moreover, no changes in the shape of the timbal were detected during electrical tensor nerve stimulation, although some small movements of the abdomen relative to the thorax were observed.

Tibicina quadrisignata. In the beginning of the calling song sequences there is an increase in the amplitude of the sound (cf. Fig. 19 A). The inward movement of the timbal generates a series of sound pulses correlated with the buckling of the sclerotized ribs of the timbal while only a soft sound is produced when the timbal springs out (Fig. 19 A).

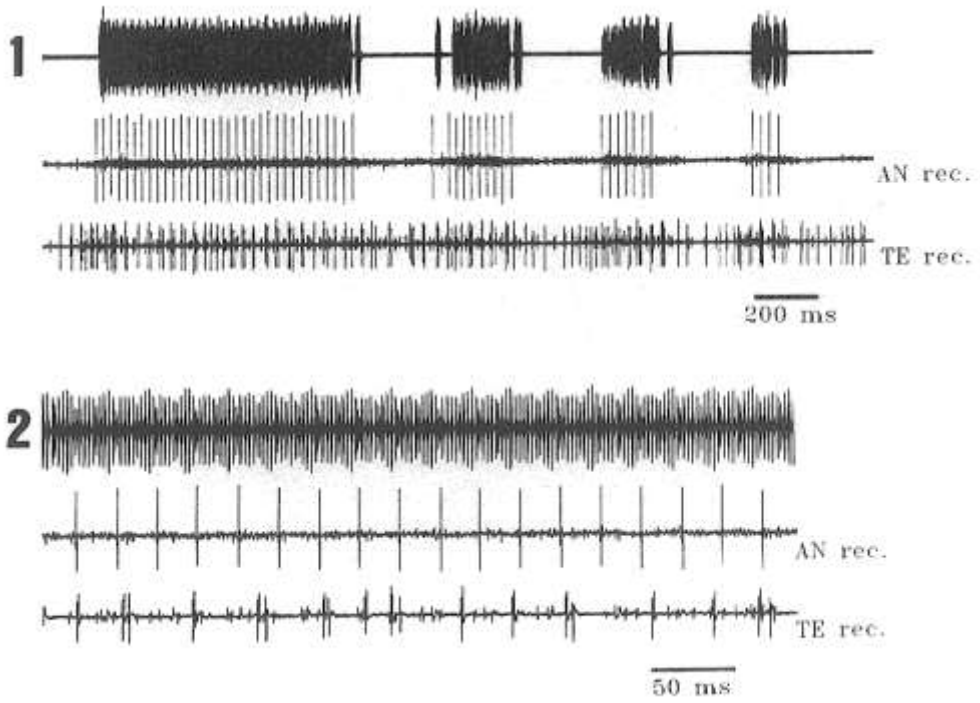


Figure 55 - Auditory nerve and tensor nerve activities during the calling song of *Tibicina quadrisignata* elicited by electrical brain stimulation.

The brain stimulation failed to elicit the amplitude increase at the beginning of the sound sequences, which, in calling animals, is correlated with an extension and elevation of the abdomen. These abdominal movements were not induced by the electrical brain stimulation.

During sound generation there was an increase of activity on the tensor nerve (Fig. 55). However, a clear correlation with the timbal motoneuron activity was not seen (Fig. 55-2).

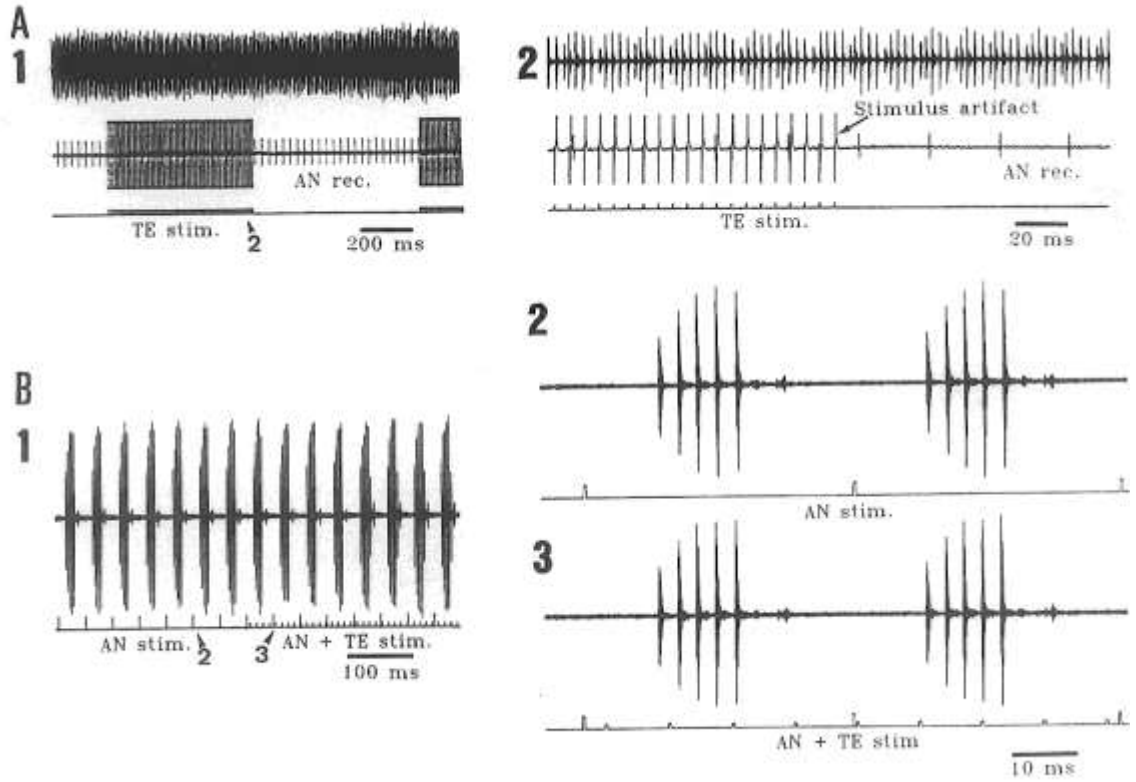


Figure 56 - Effects of electrical stimulation of the tensor nerve in *Tibicina quadrisignata*, both during singing elicited by brain stimulation (A) and in the sound generated with electrical stimulation of the auditory nerve (B). The effects were small or inexistent.

Electrical stimulation of the tensor nerve did not produce any clear effects neither during singing obtained with brain stimulation (Fig. 56 A) nor during electrical stimulation of the auditory nerve (Fig. 56 B). Moreover, there were no apparent changes on the timbal shape induced by stimulation of the tensor nerve.

The number of timbal ribs involved in the generation of the sound pulses depended on the rate of stimulation of the auditory nerve (Fig. 57). Up to 5-6 ribs could be buckled by progressively increasing the frequency of stimulation of the auditory nerve.

Discussion. The objective of this work was to elucidate if the variations on the sound amplitude and time shifts measured during singing, and not correlated with modifications on the timbal muscle EMGs, could be attributed to the action of the tensor muscle.

All experiments in this investigation did support the involvement of the tensor muscle as responsible for creating such amplitude and time modulations in *Tett. argentata/atra*, *Tett. josei* and *Tymp. gastrica*, but not in *C. barbara lusitanica* and *Tib. quadrisignata*. a) In the first three

species there was a clear correlation between the activity recorded on the tensor nerve and the timbal motoneuron spiking as well as with the sound generated during the calling song. These characteristic modulations were prevented by lesioning the nerve. b) Stimulating the tensor nerve with an appropriate rate while the males were singing or during stimulation of the auditory nerve induced similar variations on sound amplitude and latencies as observed in the calling songs. For instance the latency of the IN pulse changed in both these two experiments by about 2 ms, 5 ms and 6 ms in *Tett. argentata/atra*, *Tett. josei* and *Tymp. gastrica*, respectively. Moreover, the correlations found between sound pulse amplitudes and latencies were essentially similar to the calling song ones (compare Figs. 35, 42, 50 with Figs. 23, 25, 27, respectively; note that the

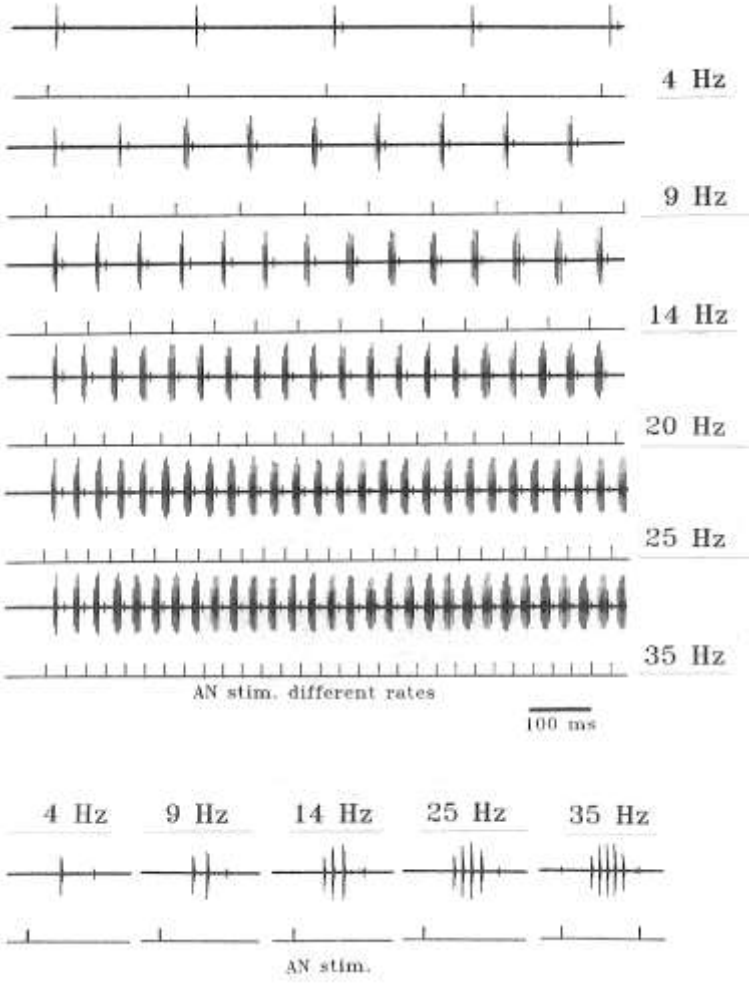


Figure 57 - Effects of increasing the rate of the electrical stimulation of the auditory nerve on the sound pulses generated during each timbal cycle by a male of Tibicina quadrisignata. The number of sound pulses, correlated with the number of timbal ribs buckled, increased with increasing rates of auditory nerve stimulation.

latencies were measured relative to the timbal muscle EMG in the calling song and from the electrical stimulus of the auditory nerve on the tensor experiments). c) The same variations of the amplitude of the sound pulses could be induced mimicking the tensor contraction by mechanically pushing on the tensor sclerite. d) Measurements of the force needed to buckle the timbal inward

indicated modifications of the timbal stiffness caused by the tensor nerve stimulation. This force increased progressively in the Tettigetta species but it decreased in *Tymp. gastrica* while in all three species the rate of the tensor nerve stimulation was increased. e) Finally, the convexity of the timbal was modified by electrical stimulation of the tensor.

However, different mechanisms were found as far as tensor muscle action was concerned. In both Tettigetta species the contraction of the tensor muscle caused an increased convexity of the timbal (Figs. 37 B, 44 B), thereby modifying its mechanics in the same way as previously suggested by Pringle (1954) and Simmons and Young (1978) for different cicadas although by a different mechanism. Here the contraction of the tensor muscle enhanced the convexity of the timbal, leading to louder sound pulses, whereas in the previously described mechanisms it is reported an increased stiffness caused by increased tension of the timbal. The increased convexity of the timbal lead to an increase in sound pulse amplitude (Figs. 33 A, 40 A) concomitant to an increase of the latency from the timbal motoneuron spike or electrical stimulus of the auditory nerve to the sound pulses (Figs. 33 B, 40 B). This was probably due to a longer time needed by the timbal muscle to develop more force which was necessary to overcome an increased convexity of the timbal and thus its stiffness. At the same time there was a shortening of the IN-OUT interval (cf. Figs. 32, 39), that could be expected from an increased elastic restoring force of the timbal caused by an enhancement of its convexity. In *Tymp. gastrica* the contraction of the same muscle achieved the opposite results, however differently from what was found in Tibicen linnei (Hennig et. al. 1994a). In *Tymp. gastrica* there was a flattening of the timbal (Fig. 52 B) accompanied by a reduction of the IN pulse amplitude and latency (Fig. 48 A,B). Correspondingly, the interval between the IN and the OUT pulses increased (Fig. 47), as was expected from a reduction of the curvature and the stiffness of the timbal. Moreover, while in both Tettigetta species it was possible to obtain a progressive increase of the effects, also seen in the calling song and likely linked to a gradual contraction of the tensor muscle, in *Tymp. gastrica* there was a sudden shift between two conditions. This abrupt modification of the system was also confirmed by the experiments where the tensor sclerite was progressively pushed inwards mechanically, mimicking the effect of the contraction of the tensor muscle, and by measurements of a spring force load needed to buckle the timbal. The precise mechanism by which the timbal

jumps between these two allowed timbal conditions in *Tymp. gastrica* was not understood, although one could see this sudden modification when controlling the posture of the timbal by sight while electrically stimulating the tensor nerve or progressively pushing on the tensor sclerite. It is likely that this effect is caused by bistable characteristics of the timbal frame around the joint-like point (Fig. 52 B).

In contrast with the findings on these three species, on *C. barbara lusitanica* and *Tib. quadrisignata* the modulations of the song did not appear to be created by the action of the tensor muscle. In both species the experiments involving electrical stimulation and lesions of the tensor nerve failed to induce large changes on the amplitude of the sound produced. There was also no clear correlation between the activity recorded in the tensor nerve and the activities of the timbal motoneurons. From the comparative morphology of the cicadas studied here, one would also expect a more important role of the tensor muscle on the modification of the sound generated in *Tett. argentata/atra*, *Tett. josei* and *Tymp. gastrica*. In these three species the tensor muscles are comparatively larger than in *C. barbara lusitanica* and *Tib. quadrisignata* (cf. Fig. 7), and their tensor sclerites may support larger movements as well.

In *Tib. quadrisignata* the increase in the number of ribs buckling with an increasing rate of electrical stimulation of the timbal motoneuron within the auditory nerve (Fig. 57) suggests that the timbal muscle needs a continuous stimulation at a certain rate in order to be able to move the timbal with the normal pattern. An increase in the number of ribs producing sound was also observed at the onset of the calling song, but this effect did not seem to be caused by an increase in the muscle contraction rate (page 107), which was already at around 50 Hz. Instead, the number of ribs buckled might be dependent on modifications on the timbal muscle, such as an increased tension induced by successive stimulation of the muscle, allowing a proper functioning.

The type of contraction response found for the tensor muscle on the cicadas studied here was not the same as describe by Hagiwara (1955), Simmons and Young (1978) and Hennig et. al. (1994a). These authors found that in some cicadas the tensor muscle undergoes a tonic contraction when the tensor nerve is stimulated by a train of electrical pulses, and so modifying the tension of the timbal. Moreover, Simmons and Young (1978) report that in the Australian

cicada Cystosoma saundersii a single stimulus applied to the tensor nerve produced little or no contraction of the tensor muscle. Contrasting with these previous findings, the analysis of the relative timing between the stimulation of the tensor and auditory nerves showed that just one electrical stimulus applied to the tensor nerve within a certain time window was enough to elicit strong effects on the sound pulses in all three *Tett. argentata/atra*, *Tett. josei* and *Tymp. gastrica* (Figs. 36, 43, 51). This data points to a phasic contraction of the tensor in these species. However, the experiments subjecting the timbal to an increasing spring force load during tensor stimulation in the *Tettigetta* indicated a gradual rise in the force needed to buckle the timbal inwards dependent on the tensor nerve stimulation rate (Figs. 37 A, 44 A), and thus suggesting also a tonic effect. The relatively slow development of the full effects induced by the tensor nerve stimulation on the sound pulses, observed on *Tett. josei* (Fig. 40), also speaks for a tonic effect of the tensor muscle. Finally, if the tensor contraction was purely phasic one would expect much larger standard deviations on the amplitude and latency values computed from the experiments with slow rates of tensor stimulation relative to the experiments with faster tensor stimulation rates, which was not the case (cf. Figs. 34, 49). Since the tensor muscle is innervated by several motor neurons (Wohlers et. al. 1979; see also Fig. 31 where different units may be recognized on the tensor recording), it is possible that this muscle displays compound tonic and phasic contraction responses.

In *Tett. argentata / atra* the IN pulses generated by both timbals fuse in the third syllable of an echeme, despite a fixed delay maintained between the two timbal muscles EMG (cf. Fig. 22 A). The mechanism by which this is achieved is still not clear. One possibility is that one tensor contracts less than the opposite one creating different latencies for the two pulses which then superimpose.

In conclusion, the quantified effects induced by the tensor muscle contraction on the sound generated and the timing of its activity are sufficient to explain the characteristic variations measured on the calling songs of *Tett. argentata/atra*, *Tett. josei* and *Tymp. gastrica*. Furthermore, in these species an intact tensor system is necessary to generate a normal calling

song pattern. However, In *C. barbara lusitanica* and *Tib. quadrisignata* other mechanisms must be responsible for the variations observed in the song.

Additionally, despite a principally similar structure of the sound producing apparatus, the effects of the tensor muscle contraction may be very different in different cicada species.

5b. Role of the abdomen

Introduction. Apart from the action of the tensor muscle, other factors influencing the sound production were recognized as well. These include the control of the position of the abdomen, changing the gap between abdomen and opercula and modulating the radiation through the tympana, and the distension of the abdomen mostly occupied by the air sacs and supposed to work as a resonant chamber at least in some cicadas (e.g. Pringle 1954; Fletcher and Hill 1978; Young 1990).

In *Cicada barbara lusitanica* and *Tibicina quadrisignata* there were no clear effects on the sound induced by the electrical stimulation of the tensor muscle, that could explain the modulations of the songs. A simple experiment was then performed to check the influence of the abdominal posture on the sound produced. This was accomplished manipulating the abdominal position after cutting the thick abdominal nerves and recording the sound generated by electrical stimulation of the timbal motoraxons.

Results. The results are summarized on Figures 58 and 59, and shall be considered preliminary.

In both species there was an increase of the sound produced while extending and lifting the male abdomen. Nonetheless, in *C. barbara lusitanica* the syllable pattern induced during the experiments was not the same observed in the calling song (cf. Figs. 9 A3, 54 A1). The amplitude differences caused by the abdominal manipulations are clearly seen on the oscillograms (Figs. 58 A, 59 A). There was, however, a basic difference in the variation of the sound spectra in both

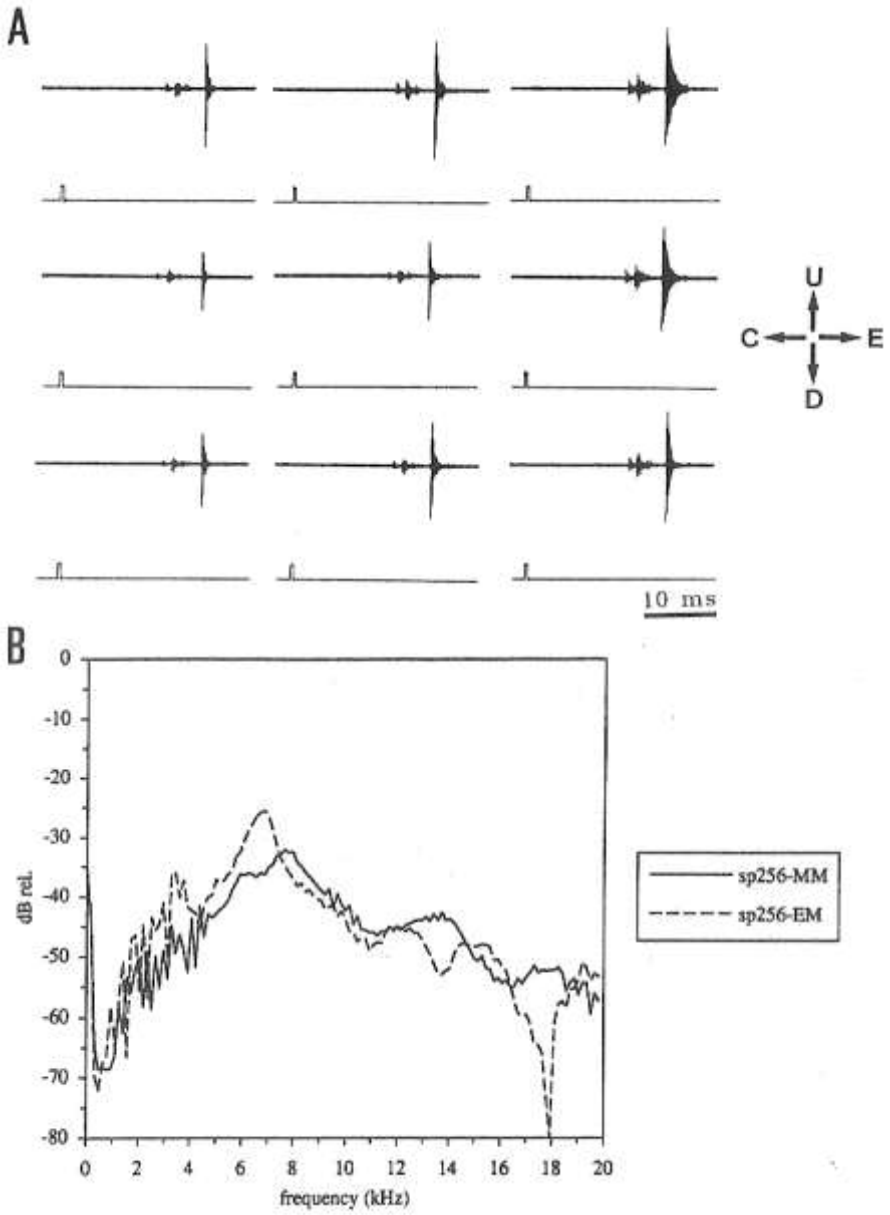


Figure 58 - Effects of the posture of the abdomen on the oscillograms (A) and the spectra (B) of the sound pulses generated by a male of *Cicada barbara lusitanica* with electrical stimulation of the timbal nerve. C compressed, M middle, E extended, U up, M middle, D down.

species. In *C. barbara lusitanica* the extension of the abdomen caused an amplitude increase at frequencies between 2-4 kHz (reaching about 10 dB around 3-4 kHz), and another larger amplitude increase around 6 kHz, where a clear spectral peak arose (Fig. 58 B).

In *Tib. quadrisignata* an extension of the abdomen caused a shift of the spectral peak of the sound pulses of about 3 kHz towards lower frequencies and a concomitant increase of the sound intensity of approximately 20 dB (Fig. 59 B). With full abdominal extension this peak was centred at about 8 kHz and thus more in accordance with the calling song spectrum (cf. Fig. 20 A).

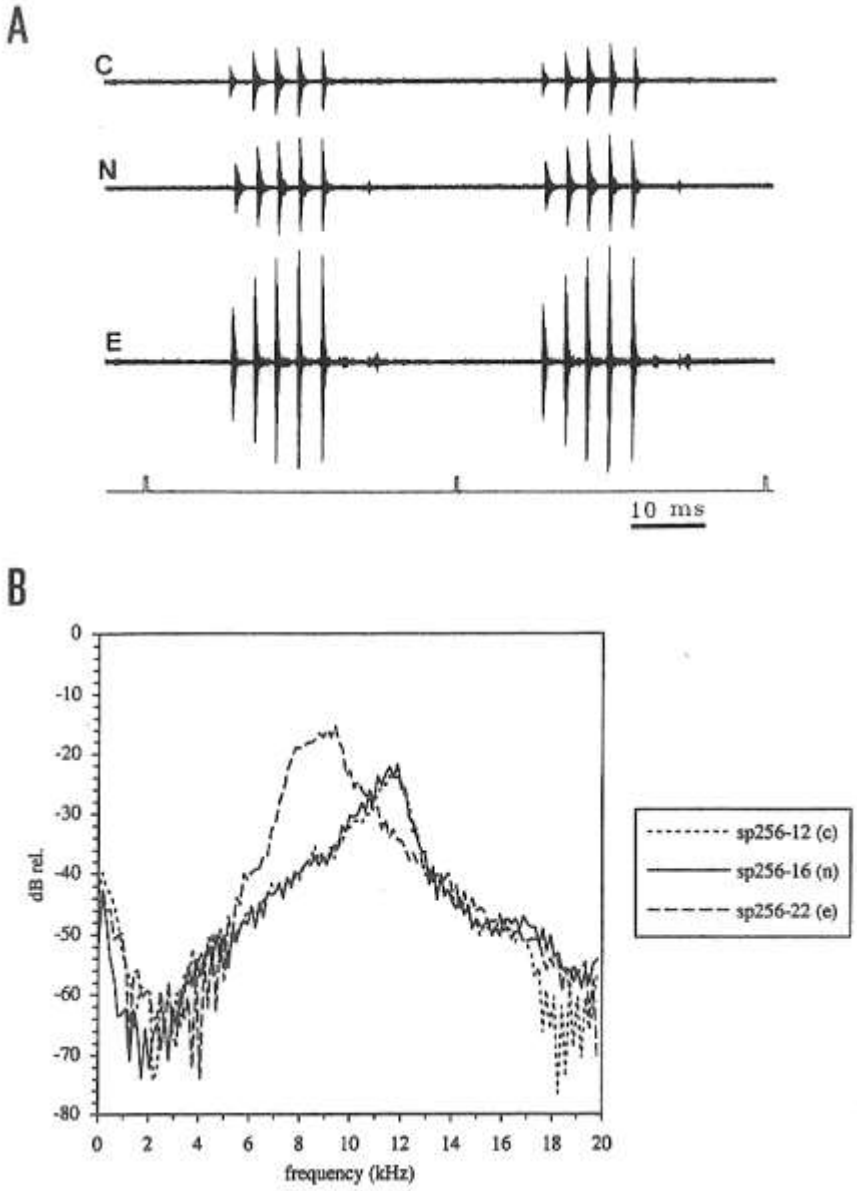


Figure 59 - Effects of the posture of the abdomen on the oscillograms (A) and spectra (B) of the sound pulses generated by a male of *Tibicina quadrisignata* with electrical stimulation of the auditory nerve.

Discussion. Although these manipulations clearly demonstrated that changes on the abdominal position and its extension kept by the males could induce large variations on the sound generated, experiments to study the biophysical mechanisms underlying the observed modifications were not conducted. Possible explanations for a relationship between the abdominal position and the amplitude and frequency spectrum of the sound pulses include: a) An enlargement of the internal volume of the abdominal cavity might change the acoustic properties of the system. This could change or sharpen the tuning of the sound spectrum as described for resonating systems (e.g.

Pringle 1954; Fletcher and Hill 1978; Young and Josephson 1983b); b) the increase of the radiation through the thin abdominal wall on *C. barbara lusitanica* with increasing surface allowed by the extension of the abdomen which also exposes the intersegmentary membranes (see discussion below about *Tymp. gastrica*); and c) the increase of the sound radiated through the tympana and folded membranes allowed by the opening of the extratympanal cavity. This could influence a possible Helmholtz resonator formed by the internal male cavities and its acoustic openings (Weber et. al. 1987; Young 1990; Bennet-Clark and Young 1992). A further indication that the folded membranes and the tympana are relevant for the sound radiated in *Tib. quadrisignata* came also from an experiment where these thin structures were sequentially blocked with vaseline, largely reducing the sound radiated (Fig. 60). However, this experiment is only preliminary and these results shall be observed with care since covering a structure not only prevents or greatly reduces its radiation but may also modify the acoustic properties of the whole abdominal air cavity system.

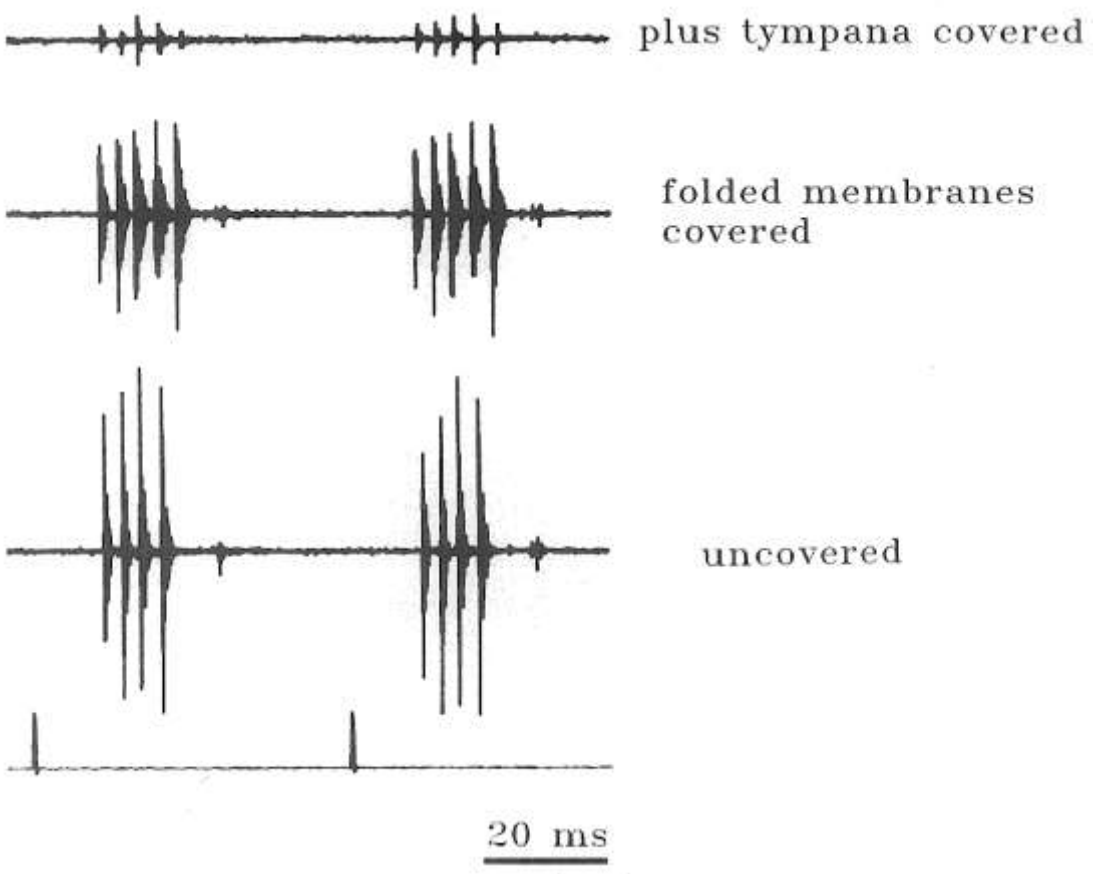


Figure 60 - Effects of sequentially blocking the folded membranes and the tympana on the sound pulses generated by *Tibicina quadrisignata* during electrical stimulation of the auditory nerve.

6. Song radiation in *Tympanistalna gastrica*

Introduction. Most cicadas elevate their bodies while singing, probably to reduce vibration damping and improve radiation efficiency. Another common behavioural feature is the extension of the abdomen, which may sometimes be extreme as in Lembeja brunneosa (Moulds 1975). It has been argued that this action could help tuning the song and allow resonances of the air inside the hollow abdomen that could be important in increasing the radiated sound intensity (Pringle 1954; Moore and Sawyer 1966; Simmons and Young 1978; Fletcher and Hill 1978; Young and Josephson 1983b). In contrast, Aidley (1969), working with Fidicina rana Walker, reported some evidence that the abdomen does not act as a resonant chamber in this species.

Many cicadas perform vertical movements of the abdomen while singing, periodically increasing the gap between abdomen and opercula, and thus widely opening the extratympanal cavity. The result is an amplitude modulation of the song pattern. Good examples are *Lyristes* (= *Tibicen*) *plebejus* (Scop.) (Popov 1969), species of *Magicicada* (Alexander 1957; Weber et. al. 1987) and *Tibicina quadrisignata* (Fonseca 1991). It has been suggested that such cicadas radiate sound through the tympana (Weber et. al. 1987). Young (1990) working with *Cyclochila australasiae* Donovan and *Macrotristria angularis* Stal came to the same conclusion after he found that sounds measured with a probe microphone in front of the timbals during distress singing were 9 dB below the values near the tympana. He modelled the system has an Helmholtz resonator, and his findings were corroborated by Bennet-Clark and Young (1992). In these species, as well as in many other cicadas, the male tympana are much larger than those of the females. In contrast, in the Australian cicada Cystosoma saundersii (Westw.), males and females have tympana of similar size and the males do not lift the abdomen during singing (Simmons and Young 1978). It was suggested that in this species with unusual low-frequency sound the abdomen acts as a resonant sound radiator (Simmons and Young 1978; Fletcher and Hill 1978). Other insights come from anatomical features. In cicadas supposed to radiate sound through the tympana, the abdominal wall is relatively thick, and the only thin membranes opening directly into the abdominal cavity are the tympana and the folded membranes.

Since males of different species can be surprisingly different in size, properties of the abdomen walls and tympana, dimensions of internal air sacs, size and form of opercula and timbal covers, it is very likely that the song radiation mechanisms are not similar in all species, and comparative data is therefore highly desirable. We also need to study radiation mechanisms while the animal is generating a normal song.

Tympanistalna gastrica Stal was used to study the possible role of different structures on the radiation of the calling song. The males of this small and delicate cicada have tympana 4.5 times larger than those of the females and possess a thin walled abdomen. They produce loud and soft sounds in each echeme of the calling song, which result from the contraction of the tensor muscle (see chapter 5a). For soft and loud pulses two modes of sound radiation could exist. Long lasting singing similar to normal calling song was evoked by means of preceding short electrical stimulation of the brain. This permitted to make numerous probe microphone measurements of sounds at different points close to the body, to study the effects of vaseline coverings of putative sound radiators, and to manipulate the size of the abdominal and thoracic air chambers. With the help of laser vibrometry measurements the structures which vibrate at most during normal singing were identified.

Results.

Calling song characteristics. The calling song of *Tympanistalna gastrica* consists of a succession of echemes (see description on page 81). In each echeme the first pulses (loud clicks) resulting from the inward movements of both timbals are much larger than the subsequent IN and OUT pulses (soft pulses) (Fig. 21-1). The echeme duration is variable, but even the shortest start with loud clicks.

The spectra of clicks and soft pulses are different. Lower frequencies are much better represented in clicks (Fig. 61). Their spectral peak around 5 kHz (s.d.=0.4, n=16) is usually, but not always, smaller than a second peak around 11 kHz (s.d.=0.3). In contrast, the spectrum of the soft pulses frequently has a minimum in the range 5-6.5 kHz (avg=5.9, s.d.=0.3, n=16), a small peak at about 7 kHz, and the main maximum around 13 kHz (s.d.=0.6). Around 5 kHz the

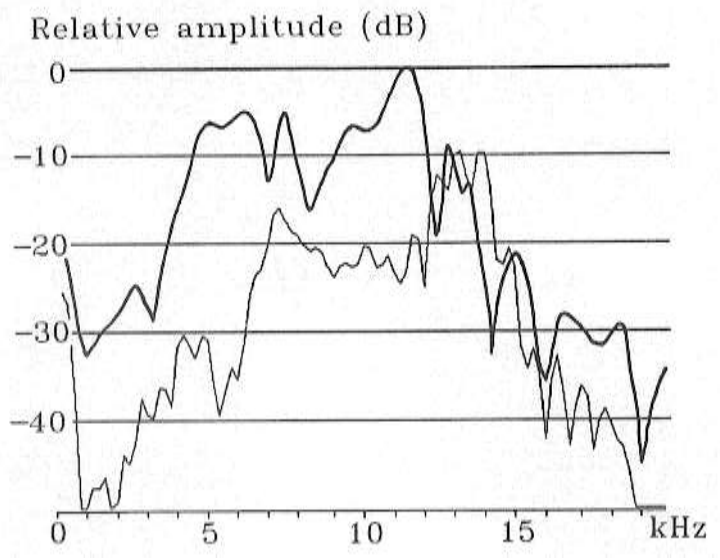


Figure 61 - Comparison between the spectra of the large sound pulses (loud clicks) in the beginning of an echeme (upper trace) and the subsequent soft pulses (lower trace), in the calling song of the cicada *Tympanistalna gastrica* recorded in the field.

sound level in clicks is generally 25-30 dB above that of the soft pulses. The sonagram (Fig. 21-2) also shows that the soft pulses have approximately the same frequency content, irrespective of being caused by inward or outward timbal movements. This is important since a complete activation cycle of both timbals was considered as the basic unit for the analysis of soft pulses.

The song obtained in the laboratory as an after effect of brain stimulation had a pattern similar to the normal calling song, and the spectra of clicks and soft pulses had the same characteristics as in the song recorded in the field.

Morphological and anatomical features relevant to the sound radiation. *Tymp. gastrica* is a small cicada. The males (Fig. 5) are 1.5-1.8cm long (measured from the tip of the head to the tip of the wings). While singing the animals extend the abdomen considerably (the length of abdomen compressed: 7 mm, s.d.=0.5; extended: 10.2 mm, s.d.=0.3, n=5), so that the intersegmental membranes are clearly seen and the abdomen tip reaches the end of the wings or extends even further. At the same time the males slightly lift the abdomen, thus opening the extratympanal cavity. The whole body is not in contact with the plant due to an extension of the legs, and the

wings are lowered and more separated than in the resting position, which gives the cicada a flattened appearence.

The ventral wall of the abdominal air sac is very thin, whereas the dorsal wall is thicker because much tissue is found between the tracheal sac and the abdominal wall. These tissues become dryer and thinner with age.

The tympana are very thin and transparent. They are much larger in the males (around 2.4 mm2) than in the females (about 0.5-0.6 mm2). The timbals are relatively thin and are not covered by tegumental folds. The timbal area reaches 2.1 mm2, excluding the thick timbal frame. The folded membrane is not as thin as in some other cicadas with thick abdominal walls.

A large hollow tracheal sac is found inside the male body (Fig. 5 B). The thoracic part of it is located anterior to the timbal muscles. The timbals and the folded membranes are installed in its wall. The abdominal part of the tracheal sac is closed by the tympana. The volume of the abdominal cavity depends on the position of the abdomen. In the maximally extended state it is about 0.1 cm3. The thoracic cavity is much smaller, about one tenth of the abdominal one (cf. Fig. 5). Both compartments of the air sac are connected by a passage between the timbal muscles and are open to the exterior through the 3rd spiracles.

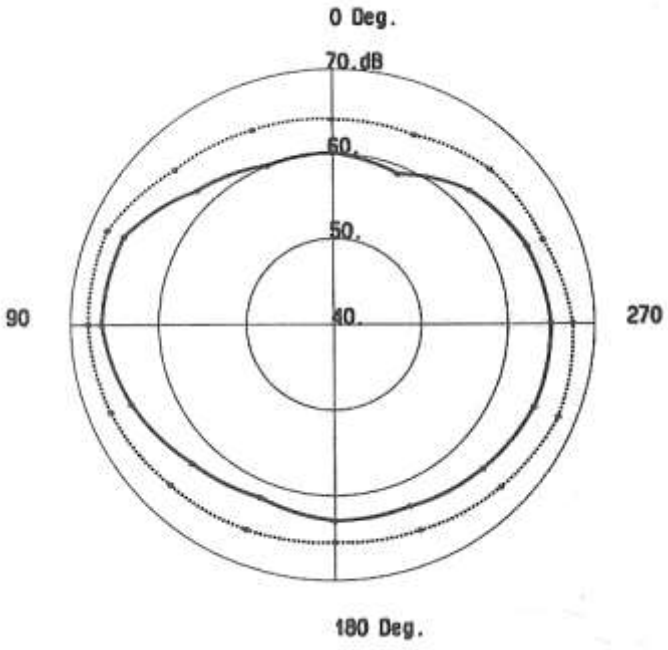


Figure 62 - Sound radiation diagrams of the calling song of a male of Tympanistalna gastrica, measured with a 63 Hz high-pass filter (dotted line) and a 1/3 octave band-pass filter centred at 13 kHz (solid line), frequency corresponding to the peak of the calling song spectrum. The measuring microphone was moved along a horizontal plane around the singing male (dB values relative to 20 microPascal).

Sound radiation diagrams. A typical sound radiation diagram measured during calling song is shown on Figure 62. The measurements of the overall sound output made at 1 m from the male cicada approach an omnidirectional diagram (dotted line). However, the use of a 1/3 octave filter centred at the spectral peak of the song (13 kHz, solid line) reveal clearly more sound pressure at the sides of the animal where the timbals are located, with differences relative to the measurements in front of the head larger than 5 dB.

Probe microphone measurements during singing. The results are summarized in Figures 63 and 64.

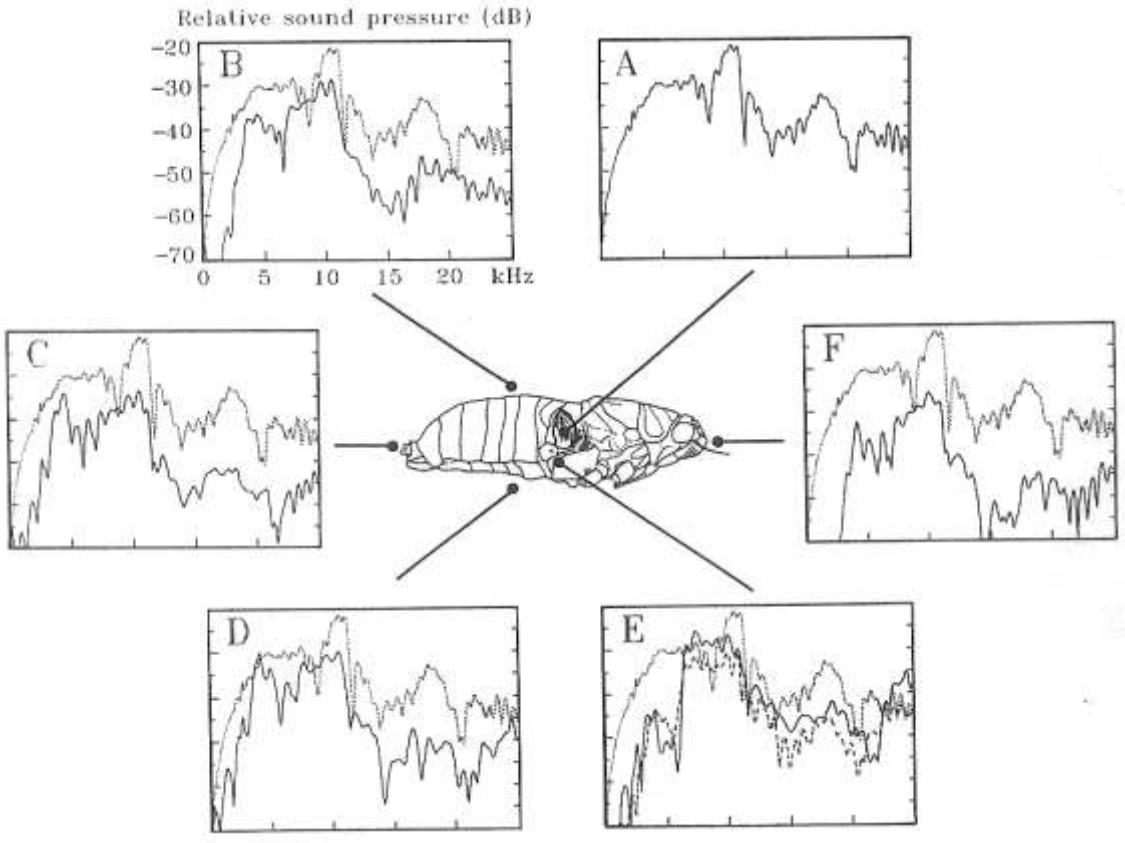


Figure 63 - Averaged spectra of loud clicks occurring in the beginning of the echemes of the song in one animal. Sound was measured with the tip of the probe microphone in different places at 1-2 mm from the surface of the cicada. For comparison the spectrum measured in front of the timbal is superimposed in all other figures (dotted line). Positions of the probe: A) Timbal; B) Dorsal mid line of the abdomen in front of the 4th abdominal segment; C) Tip of the abdomen; D) Ventral mid line of the abdomen in front of the 4th abdominal segment. E) Dashed line - in front of the operculum; solid line - same position but after removing the opercula; F) In front of the head.

For the loud clicks, the sound level recorded near the timbal was above or at the levels recorded at other positions. The low frequency region (3-9 kHz) was well represented and the main peak was around 10-11 kHz (Fig. 63 A). There is also a smaller peak at 17-18 kHz. Near

the tympanum (opercula removed) the level of most frequencies was much lower except in the range from 7 to 11 kHz (Fig. 63 E, solid line). When the probe was at the medio-ventral part of the abdomen, the spectrum of loud clicks had a pronounced peak in the 4-5 kHz region which was 10 dB above that in tympanum position and close to the spectrum measured in timbal position, and a second peak in the range 7.5-11 kHz which was a bit below the spectral line in tympanum position (Fig. 63 D). When the probe was shifted towards the tip of the abdomen, the level of both peaks was attenuated by 5-10 dB (Fig. 63 C). With the probe above the abdomen the spectrum was not very different from that in ventral position except that the 4-5 kHz peak was less prominent (Fig. 63 B). At the head of the animal all frequencies were attenuated (Fig. 63 F).

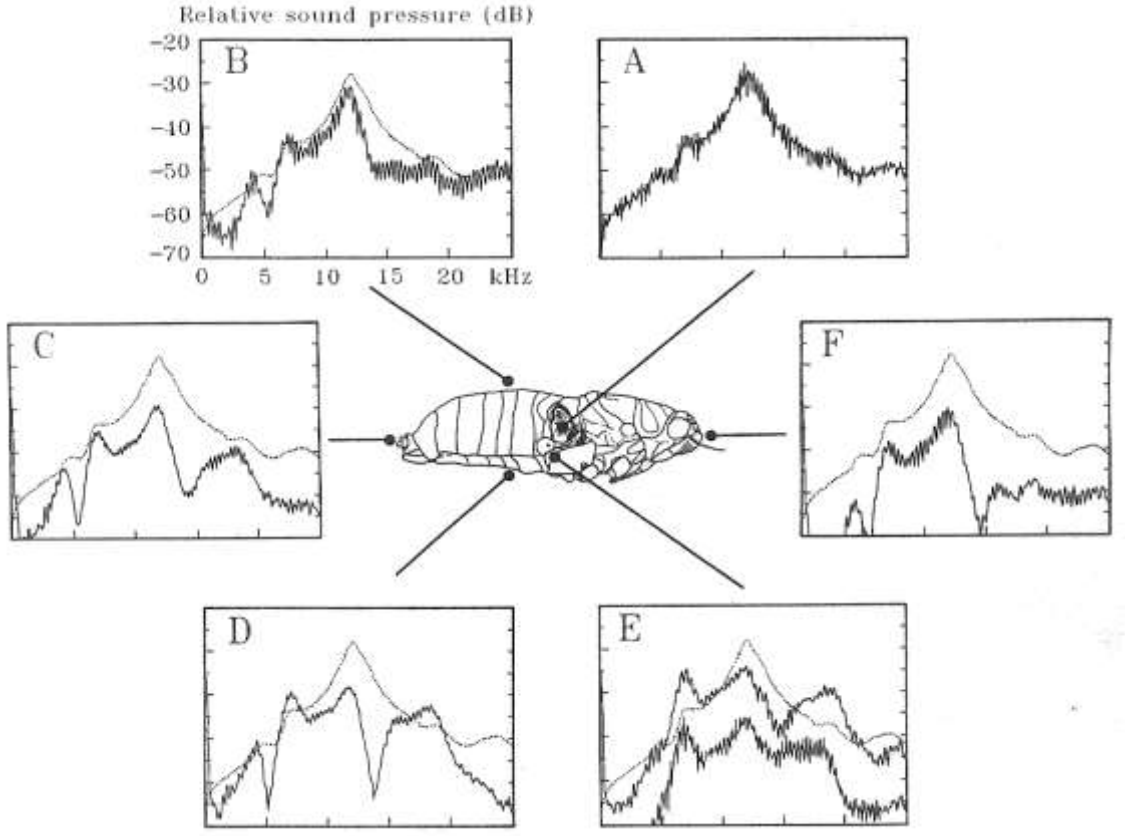
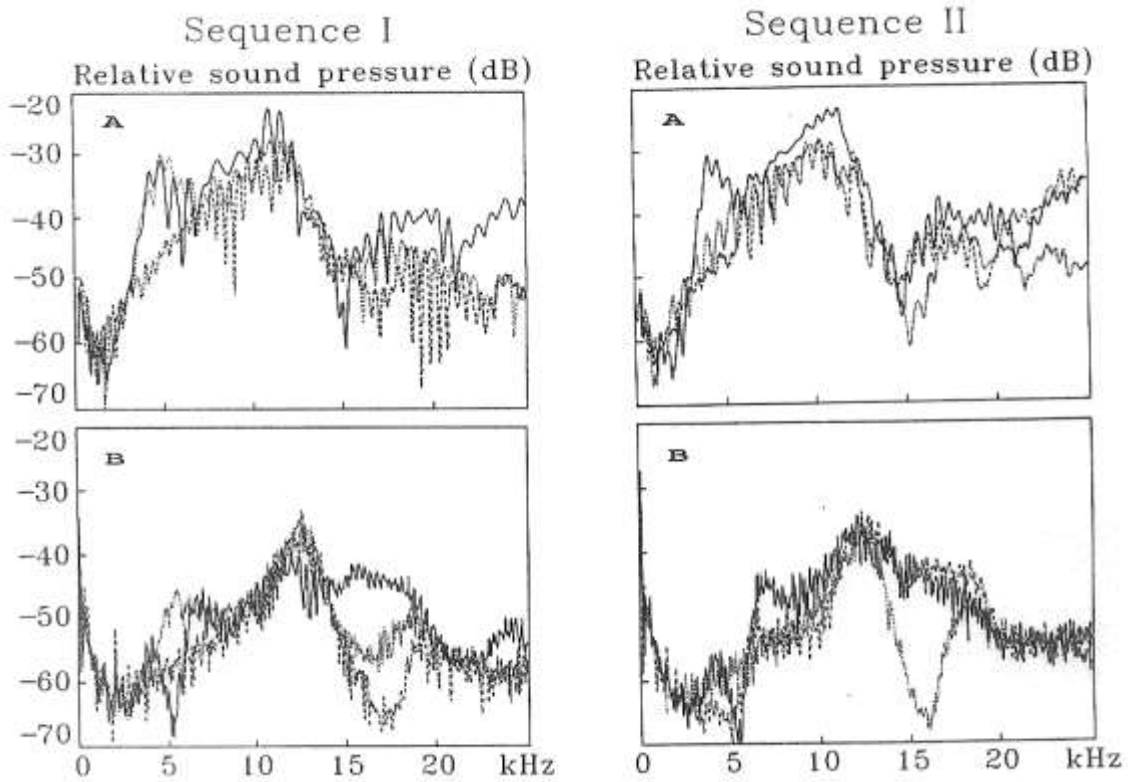


Figure 64 - Averaged spectra of the soft sound pulses in the song of the same animal as in Fig. 63. Sound was measured with the tip of the probe microphone in different places at 1-2 mm from the surface of the cicada. For comparison the spectrum measured in front of the timbal is superimposed in all other figures (dotted line). Positions of the probe: A) Timbal; B) Dorsal mid line of the abdomen in front of the 4th abdominal segment; C) Tip of the abdomen; D) Ventral mid line of the abdomen in front of the 4th abdominal segment. E) lower solid line - in front of the operculum; upper solid line - same position but after removing the opercula; F) In front of the head.

The picture was different with soft pulses (Fig. 64)[4]. The spectrum measured near the timbal had only one well expressed peak around 12.5 kHz (Fig. 64 A). In front of the tympanum the spectrum had three peaks, around 7, 12 and 19 kHz, the first and the third exceeding the level of the timbal spectral curve when the opercula were removed. Removal of the opercula resulted in approximately 10 dB elevation of the whole spectral curve (Fig. 64 E). The spectra of the soft pulses recorded at the medio-ventral part of the abdomen (Fig. 64 D), abdomen tip (Fig. 64 C) and in front of the head (Fig. 64 F) had two deep minima around 5.4 kHz and 14.5 kHz which were not an artifact of the analysis since they were present in the spectrum of a single pulse. The level in all these spectra was below the level in the spectrum measured at the tympanum and had the same peaks. The timbal radiation predominated above the abdomen, since the timbals are situated dorsally and are oriented backwards (Fig. 64 B). Here only one minimum was present around 5.4 kHz and the rest of the spectral curve was very close to the timbal one.

The effect of removal of the opercula was prominent only for measurements near the tympanum and was negligible in other positions.



[4] The measurements were not reproducible in details of the fast oscillations (smoothness) of the spectral lines. This is probably created as an artifact of the analysis involving the calculation of spectra from segments with more than one sound pulse. These fast oscillations were nearly absent in the spectrum of a single pulse.

Figure 65 - Effect of covering several structures on the sound produced during singing, following two covering sequences. A) averaged spectra of loud clicks occurring in the beginning of the echemes. B) Averaged spectra of the subsequent soft sound pulses. Sequence 1: initial condition with the opercula removed - solid line; Tympana and folded membranes covered - dotted line; plus the whole abdominal surface covered with vaseline - dashed line. Sequence 2: initial condition with the opercula removed - solid line; whole abdominal surface covered with vaseline - dashed line; plus tympana and folded membranes covered - dotted line.

Covering experiments. Figure 65 presents the spectra of loud clicks and soft pulses obtained with two covering sequences. I was aware that covering a structure with vaseline (= loading it) might change the acoustics of the system in an unpredictable manner. Nevertheless, using two covering sequences reduced the risk of misinterpretation, and together with other experiments, these manipulations might help to understand the role of different structures in sound radiation during singing.

Covering the abdomen with vaseline resulted in considerable attenuation (about 15 dB) of frequencies around 5 kHz in the spectrum of loud clicks for both sequences (Fig. 65-IA, 65-IIA). At least half of this effect was obtained when only the ventral side of the abdomen was covered. Covering the tympana did not influence the 5 kHz region, but reduced the level above 15 kHz. The 11 kHz peak was not much reduced by covering of the several structures. Covering the folded membranes had no considerable effect. In two of the six experiments when the 3rd spiracles were blocked after the abdomen and tympana it was observed an additional small reduction at frequencies below 10 kHz. This could be related to the fact that during singing there is a strict synchronization between the opening of the 3rd spiracles and the generation of the echemes, easily observed when singing activity is monitored under a stereomicroscope.

In contrast to loud clicks, for the soft pulses the effects were dependent on the covering sequence. In sequence 1, covering the tympana increased the sound level around 5 kHz by more than 20 dB, where the dip disappeared, supporting the idea that the dip is the result of destructive interaction of sounds from different radiators, whereas high frequencies around 17 kHz were attenuated by approximately 10 dB (Fig. 65 IB). The peak at 12.5 kHz was sometimes, but not always, slightly increased. Subsequent covering of the abdomen consistently reduced the sound level around 5 kHz by approximately 10 dB and produced additional attenuation around 17 kHz. Again, at least half of the effect could be obtained after covering only the ventral abdominal surface.

However, covering the abdomen first (sequence 2, Fig. 65-IIB) did not significantly change the spectrum of the soft pulses around 5 kHz, where the dip was maintained, apart from a small reduction around 4 kHz. Frequencies between 6 and 11 kHz were also attenuated. Subsequent covering of the tympana resulted in strong, sometimes dramatic, attenuation around 16 kHz and about 10 dB increase of levels around 5 kHz, where the dip disappeared. No difference was found at the 12.5 kHz peak.

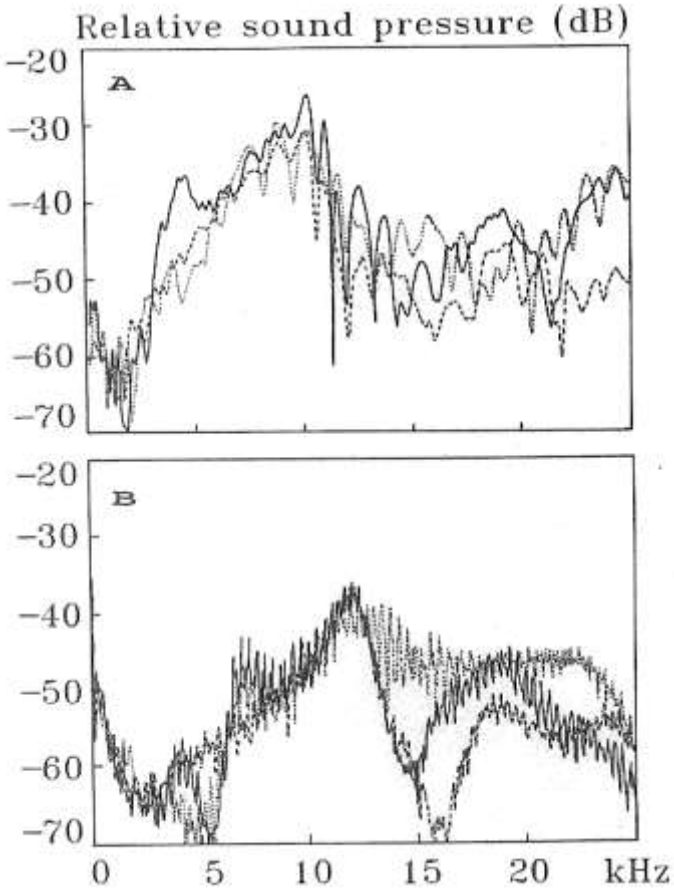


Figure 66 - Effect of the shape/volume of the abdomen on the radiation of the song. A) Averaged spectra of loud clicks; B) Averaged spectra of the soft pulses. Abdomen extended - solid line; abdomen compressed - dotted line; abdomen extended but tympana, folded membranes and abdominal surface covered with vaseline - dashed line.

Manipulations of the internal air cavities. Figure 66A demonstrates the spectra of loud clicks generated with the abdomen extended, compressed, and again extended, but this time with the folded membranes, tympana and abdomen covered with vaseline. Compression of the abdomen caused a clear attenuation of sound around 5 kHz. This effect could be a result of the closing of the folded membranes, a reduction of the exposed abdomen surface and a reduction of the abdominal cavity volume in the compressed position. Comparing this spectrum (dotted line) with one obtained after the above mentioned coverings (dashed line), one can find close similarity

below 14 kHz, suggesting that differences in the 5 kHz region in extended and compressed state of the abdomen were mainly due to the reduction of the exposed surface.

Again, the picture for soft pulses was different (Fig. 66 B). Now the effect of compressing the abdomen was only slight pressure decrease at 4 kHz and an increase of the levels above 13 kHz.

Experiments where the anterior internal cavity was reduced by injecting vaseline showed that its volume is not important for the tuning of the timbals. The spectra before and after reducing this cavity down to about one quarter, when the connection to the abdominal part of the air sac was practically closed also, were very similar in their pattern (Fig. 67).

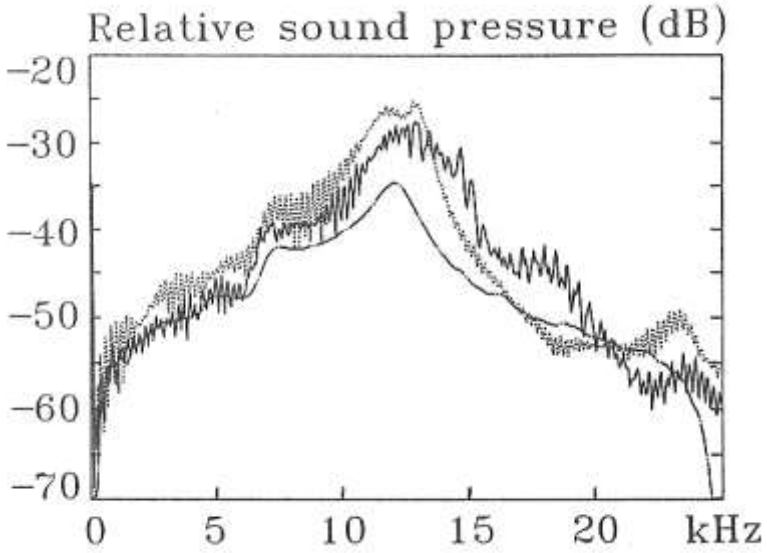


Figure 67 - Effect of reducing the volume of the anterior internal chamber on the spectra of the soft sound pulses. Initial condition - solid line; spectrum after the injection of some vaseline into the chamber - dotted line; spectrum when approximately 3/4 of the anterior chamber was filled up - dash-dotted line

Laser measurements. The timbal vibrations induced by external sound stimulation had a prominent peak at about 12.5 kHz (Fig. 68) which corresponds well to the spectral peak of the soft pulses of the song. Large vibrations were also found on the ventral surface of the abdomen. Their amplitude was much bigger than that of the dorsal wall vibrations. This was not surprising since the dorsal wall is much thicker, being lined by internal tissues.

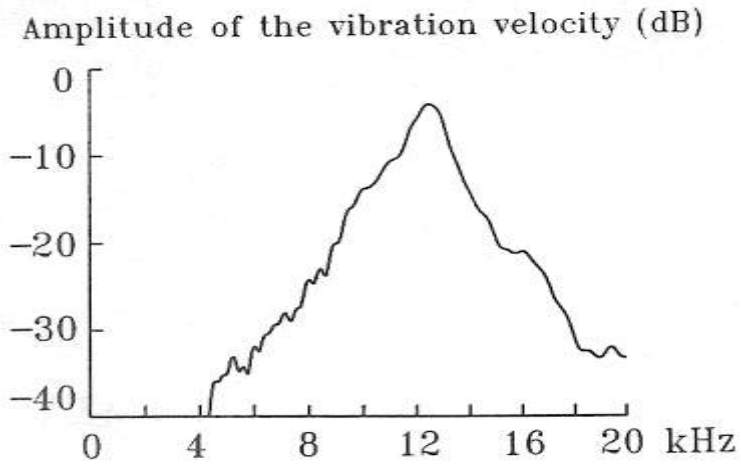


Figure 68 - Frequency response of the timbal membrane with external ipsilateral sound stimulation. The curve was scaled to 80 dB SPL; 0 dB corresponds to 3.2 mm/s.

Precise LDV measurements during singing appeared to be practically impossible with the method used because of too large amplitude and/or complexity of movement of vibrating structures. In most cases either the input was overloaded or the laser beam became out of axis due to movements of the structure under measurement. Nevertheless some remarks can be made: 1) the largest vibrations were found in the timbals and tympana; 2) vibrations of the ventral abdominal wall and folded membranes were much smaller; 3) Practically the whole animal was vibrating during singing, and the smallest amplitudes were found in the thorax and at the head. The vibrations measured on the holder were much smaller than any animal vibrations.

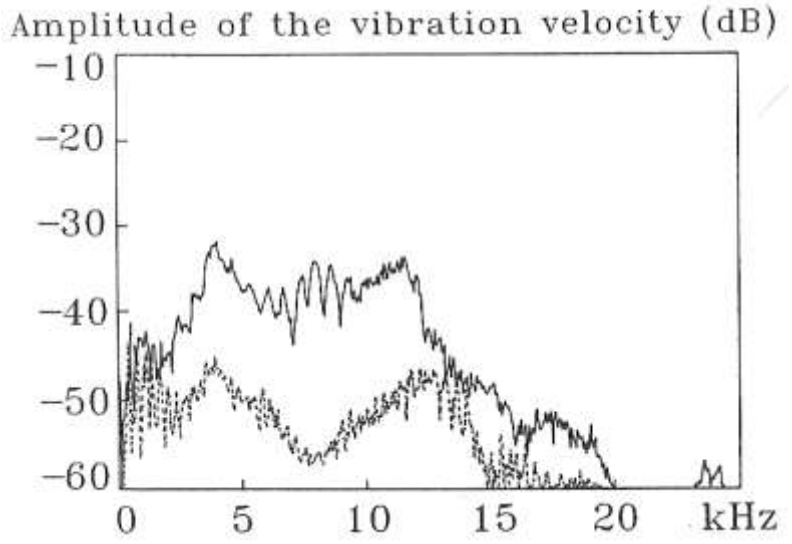


Figure 69 - Vibrations measured on the ventral surface of the 3rd abdominal segment during singing. Loud clicks - solid line; soft sound pulses - dashed line.

Figure 69 demonstrates the spectra of the vibrations of the medio-ventral surface of the 3rd abdominal segment during the generation of loud clicks and soft pulses. Note that the amplitudes are 10-18 dB higher in the range 3-12 kHz during click production. There was a good

correlation between the spectral peaks of the abdominal vibrations and the loud click sounds; vibrations were well represented both in low frequency (around 4 kHz) and high frequency (around 11 kHz) regions.

Discussion. The two components of *Tympanistalna gastrica* calling song - loud clicks and soft pulses - appeared to be very different in their spectral content. The former have two peaks around 5 kHz and 11 kHz, respectively, and the latter has the main peak at 12-13 kHz and a secondary peak around 7 kHz which is usually 6-10 dB lower. This is an indication that the sound production mechanism can work in different modes. In this cicada, as in many others (Popov 1990b), the hearing organs are most sensitive at low frequencies around 3-4 kHz with a roll off towards higher frequencies (cf. Fig. 98, page 231). Thus, the loud clicks of the calling song which are rich in low frequencies, can better serve the distant communication of males and females, whereas the high frequency soft pulses are probably used mostly at close range, as described for Cicadetta sinuatipennis Osh. (Popov 1981). However, in the latter case loud clicks are produced by the wings and soft sounds by the timbals.

The experimental data presented here indicate that several structures - principally the timbals, the tympana and the abdomen wall - are involved in the radiation of the calling song, and their relative importance is different during the production of loud clicks and soft pulses. The probe microphone measurements shall be reliable enough because, even if the sound recorded at any point is a result of complex interaction of waves generated by different sources, the output of the nearest structures is expected to be emphasized. However, this is probably not true for the probe position in front of the head. Besides, at some frequencies there are signs of possible destructive interaction of sound from different sources in other positions too (see, for example, Fig. 64 D,E,F). Nevertheless, these measurements suggest that all three radiators mentioned are used for soft pulses, but for different spectral ranges. The timbals seem to be more important for the radiation of frequencies around the spectral peak of the song (12-13 kHz) (Fig. 64 A); the tympana for wider range of frequencies including those below and above the timbal peak, since at their vicinity the spectrum contains three peaks (7, 12 and 19 kHz) but only two of them (7 and 19 kHz) are above the spectrum measured near the timbal (Fig. 64 E) when the opercula are removed. This does

not mean, of course, that the tympana are not radiating frequencies around 12-13 kHz. Simply that they seem to be more efficient radiators of sound at 7 and 19 kHz peaks than are the timbals. The wall of the abdomen gives small additional radiation at lower frequencies around 4 kHz. In favour of such an interpretation speaks also the measured frequency response of the timbals to external sound stimulation which has only one clear maximum at 12-13 kHz (Fig. 68), the measured radiation diagrams (Fig. 62) and the results of the covering experiments (Fig. 65). During the production of soft pulses, the level of the timbal peak was not significantly changed by blocking the folded membranes, the tympana and the abdomen, whereas lower and higher frequencies were attenuated.

During the production of the loud clicks the abdomen seems to play a major role in the radiation of frequencies around 4-5 kHz, since covering this structure greatly reduced the sound intensity in this range, irrespective of the state of the tympana (covered or free) (Fig. 65 A). This interpretation is supported by probe microphone recordings in the abdominal region, especially on the ventral side, where a prominent peak at 4-5 kHz was found (Fig. 63 D). Although this peak was at or slightly below the level of these frequencies in measurements near the timbal, it can be supposed that the abdomen is a better radiator for lower frequencies because its surface is much larger than that of the timbals. In fact, the efficiency of sound radiation should increase with the surface of the source until its size approaches the wavelength of the emitted sound (Bennet-Clark 1971; Michelsen and Nocke 1974; Michelsen 1983). Both abdomen and timbals are much smaller than the wavelength at 5 kHz (68 mm). Using approximate values of the respective surfaces (2.1x10-6 m2 for the timbals and 75x10-6 m2 for the abdomen wall) and the plot of Figure 1 in Michelsen (1983), one can find that the 5 kHz power radiated from the abdominal wall is at least two orders of magnitude above the power from the timbals[5]. Of course, this is probably simplifying the system too much, and we are also not considering the amplitude of the vibration of both structures. If it appears that the timbal vibrations at 5 kHz are much larger, then this difference in radiation power will be reduced. Anyway, the LDV measurements at hand show that the spectrum

[5] About 3 orders of magnitude is the result considering the computations using the relation 6 on Michelsen (1983). Two orders is a cautious figure considering that the abdominal surface is not overall equally thin.

of vibrations of the ventral abdominal wall has its main peak at 4 kHz and this peak is about 10 dB larger in loud clicks than in soft pulses (cf. Fig. 69).

The data led to the hypothesis that in order to activate the abdomen to radiate sound around 4-5 kHz, a threshold in the energy submitted to it by the timbals has to be reached. Support of this idea comes from the comparison of spectra of loud clicks and soft pulses in covering experiments. In sequence one, covering the tympana provoked a significant amplification around 5 kHz, where a dip disappeared (cf. Fig. 65-IB). One can think of at least two reasons for this observation: 1) the sound radiated by the tympana was interacting destructively with the sound radiated by other structures, creating a dip; 2) before covering the tympana, these very thin and elastic membranes released too much pressure and the abdomen could not be activated at these frequencies; when the tympana were blocked there was not enough pressure release and the abdomen was activated.

Morphologically, the thin wall of the abdomen, especially on its ventral surface and intersegmental membranes, is well suited for sound radiation. Blocking the tympana has a much smaller effect on loud clicks than on soft pulses in the whole frequency range but above 17 or 18 kHz, suggesting that during production of clicks the pressure release through the tympana is not enough to prevent the abdomen to be activated and to radiate low-frequency sound, radiation by the tympana becoming unimportant during the production of loud clicks.

The posture of a singing male is also consistent with the idea that abdomen and tympana are important radiators.

The reason why the spectral peaks of loud clicks and soft pulses are so different (11 and 12.5 kHz respectively) is unclear. It may be related to the mechanics of the timbals themselves, modified by tensor muscles (the data on the role of the tensor above; Pringle 1954; Hagiwara 1955; Hennig et. al. 1994a), since neither covering of other structures nor manipulations with the volume of the internal cavities or the size of the abdominal surface influence significantly the position of these peaks.

Young (1990) introduced the idea that in typical cicadas the resonant air vibrations in the abdominal cavity passively drive the tympana which are the main radiators of the sound. He modelled this system as a Helmholtz resonator, and his idea was corroborated by the detailed

study of Bennet-Clark and Young (1982). Applying their reasoning to *Tymp. gastrica*, and using the following approximate values: a) the cross sectional area of the neck approximated by the area of the tympana - about 5x10-6 m2; b) the length of the neck derived from the equivalent hole radius corresponding to the area of one tympanum, including the acoustic corrections presented in Bennet-Clark and Young (1992), and approximated by 1.5x10-3 m; and c) the air volume in the extended abdomen - around 0.1x10-6 m3, one gets the calculated resonant frequency 9.8 kHz which is not in good agreement with the spectral peaks found in the song[6]. In view of this model one would also expect to find a significant difference in the position of the peaks when the abdomen is extended and then compressed. The same calculations for the compressed abdomen (abdominal cavity is reduced to 65%) give a resonant frequency of 12.2 kHz. In the experiments such a difference was not found neither in loud clicks nor in soft pulses (cf. Fig. 66), although in the spectra of the soft pulses frequencies above 13 kHz were amplified when the abdomen was compressed. Moreover, the stimulation of the cicadas with external sound while measuring the sound pressure inside the abdomen did show that the excess sound pressure found at about 13 kHz was abolished covering the timbals, suggesting that this peak was created by the vibrations of the timbals and not by intrinsic resonant properties of the internal air sac (cf. Fig. 90). Thus the present data is not in complete agreement with the idea that in *Tymp. gastrica* the abdominal cavity is also working as a Helmholtz resonator. But this species is not typical in the sense used by Young (1990) since it possesses a thin walled abdomen. Other important differences between *Tymp. gastrica* and the two Australian species studied by Young (1990) and Bennet-Clark and Young (1992) are in the spectra of their sound signals. Since *Tymp. gastrica* has a broad band spectrum and C. australasiae and M. angularis have much narrower song spectra, different underlying radiation mechanisms may be suggested.

The results obtained in experiments with extension and compression of the abdomen point to the importance of the size of its surface in the radiation of low frequencies, and further

[6] One major difficulty in the calculation of the resonant frequency considering the system as a Helmholtz resonator resides in the estimation of the neck length since this measurement refers to the amount of air effectively displaced during the vibration. Another source of uncontrolled error arises from the fact that changing the abdomen extension we also change the gap to the extratympanal space, probably affecting the air volume corresponding to the neck of the resonator model. Furthermore, possible sources of errors relate to the estimation of the volume of the air sac effectively contributing to the supposed Helmholtz resonator since the volume of air adjacent to the walls of the cavity may be significant in such a tiny species, along with the presence of non rigid walls. So, conclusions based on these calculations should be taken cautiously and as preliminary.

suggest a less important role of abdominal resonances. Figure 66 seems to support this idea. The drop in low frequency radiation (around 5 kHz) during generation of loud clicks is probably caused mainly by the reduction in the exposed abdominal surface after retraction, since the spectra of loud clicks produced with abdomen extended or compressed but with abdominal surface completely blocked by vaseline are similar below 14 kHz. Note that the covering experiments in sequence 2 did show that the attenuation in the 5 kHz region was caused by covering the abdomen and was independent of the tympana (cf. Fig. 65).

The spectra of the sound produced by the animal while the volume of the thoracic chamber was reduced (Fig. 67) show that this cavity is not important in tuning the timbals, but it may be significant in allowing the timbals to vibrate, thus reducing damping (Aidley 1969). Instead, the timbals seem to have their own natural frequency. This view is also supported by the LDV measurements and by the covering experiments. Intrinsic vibration properties of the timbals were also found in other species (Pringle 1954; Young 1973; Simmons and Young 1978).

7. Conclusion

The requirements from an emitter in the context of an acoustic communication system, i.e. detectability and specificity, most likely led to adaptations of the sound production system of timballing cicadas. The data presented on the chapters above is now used as an approach to the basic problems outlined in the foreword: 1) To be detectable -- What are the structures involved and how do they function?, and 2) To be specific -- How does the interplay of nervous activity and morphological structure determine specificity?

1) Sound production is obtained by buckling a pair of timbals basically in the same way in all timballing cicadas. However, there is a large variability between species in the structures involved in the sound production system: timbals, tympana, folded membranes, tracheal air sac, timbal and tensor muscles, which determine differences in the sound signals produced.

The frequency spectrum of the sound signals is dependent on the acoustic properties of the structures involved in sound radiation which are driven by the timbals also radiating sound by themselves. Related species belonging to the same genus appeared to have a similar construction of their sound production structures (e.g. timbals, tympana, abdomen thickness, folded membranes) as revealed by their similar morphologies. To these related morphologies correspond similar biophysical properties revealed by similar timbal cycles and frequency spectra which largely overlap (e.g. *C. barbara lusitanica* and *C. orni*). There are examples known, however, where closely related cicada species show very different frequency contents of their songs (e.g. *Magicicada septendecim* vs *M. cassini*, Young and Josephson 1983).

At least some cicadas are able to obtain a certain frequency modulation of their sounds. Different mechanisms allowing sound frequency modulation were observed here: a) A decrease of the frequency where the spectral peak of the sound pulses occurs was observed in *Tib. quadrisignata* as a result of an abdominal extension. This is apparent comparing the spectra of sound pulses induced by electrical stimulation of the timbal motoneuron while manipulating the abdomen. The frequency variations in this species are likely to be due to different resonancies set on the air sac with different air volumes, probably by a mechanism similar to the one described by Young (1990) and Bennet-Clark and Young (1992) -- a Helmholtz resonator. b) The modification of the mechanics of the timbal (convexity, stiffness) induced by the contraction of the tensor muscle can also modify the frequency contents of the sound pulses, as is clearly demonstrated for instance in *Tymp. gastrica*. In this species the large pulses at the beginning of each echeme have both a different spectral peak and exhibit higher intensity at lower frequencies than the small pulses induced by the contraction of the tensor muscle. Such differences can be seen both in the calling song and during laboratory experiments where the tensor muscle and the timbal motoneuron were electrically stimulated. Frequency modulations using the same principle were also seen in *Tett. argentata/atra* and *Tett. josei*. c) In *C. barbara lusitanica* it was not understood how the frequency modulation seen in the songs is achieved, but it did not appear to be due to similar mechanisms as outlined before.

The sound signals of the cicadas show amplitude modulation as well, and this effect can also be produced in several different ways: a) By varying the gap between the abdomen and the

opercula as a result of vertical movements of the abdomen, which leads to changes in sound radiation through the tympana (Weber et. al. 1988; Young 1990). *Tib. quadrisignata* shows such a mechanism. b) By modifying the convexity, and therefore the stiffness, of the timbals through the contraction of the tensor muscle, as was previously suggested by Pringle (1954). The effects obtained can be either an increase (*Tett. argentata/atra*, *Tett. josei*) or a decrease (*Tymp. gastrica*, this thesis; *Tibicen linnei*, Hennig et. al. 1994a) of the amplitude of the sound pulses. c) In *C. barbara lusitanica* the large sound amplitude modulation could not be explained by the previous two mechanisms. It is possible that they modify the air pressure inside the air sac and that this affects the system in an unknown manner in order to cause the modifications in the sound amplitude.

Sound radiation has to be optimised in order to obtain loud signals with a lower energy input. Therefore, concentration of the sound energy in certain frequencies should be an advantage. This is obtained in some species by using the timbals to drive a resonant internal air cavity. Such a system was suggested by Pringle (1954) and seems to occur in several cicada species (e.g. Simmons and Young 1978; Fletcher and Hill 1978; Young 1990; Bennet-Clark and Young 1992). Young (1990) found that in some Australian species with a thick walled abdomen (*Cyclochila australasiae* and *Macrotristria angularis*) which generate relatively tuned songs, the abdomen was functioning as a Helmholtz resonator and the sound was mainly radiated through the tympana. In *Tymp. gastrica*, however, this does not seem to be the case. Instead, the sound is radiated through several structures: the timbals, which vibrate at a certain frequency, appear to be important in the radiation of this frequency which corresponds to the peak of the soft pulses in the calling song; the tympana play a role in sound radiation at frequencies below and above the timbal peak, especially during the generation of the small pulses of the song; the thin abdomen wall, which has a much larger surface than the timbals and the tympana, is important in the radiation of lower frequencies which occur mainly during the production of the large sound pulses of the song. Radiation largely by the abdomen seems also to occur in the Australian species Cystosoma saundersii, a species with a calling song with an unusual low frequency sound (800 Hz) and a large thin walled abdomen (Simmons and Young 1978, Fletcher and Hill 1978).

Thus cicadas have different possibilities of controlling to a certain extent the frequency and amplitude of their sound signals and different structures may be involved. Moreover, the biophysical mechanisms of radiation and the body structures involved can be different not only in different species but also during the production of different song components by one species. Therefore, a unifying model for all cicada species is not possible at this date.

Behavioural mechanisms may also be used to improve the range within which a sound signal generated by a male cicada would be detectable. Those may include singing from an elevated position, which is a common behaviour in cicadas, or the high mobility observed in small species when compared to larger ones. While large species often call for a long time from one point (e.g. *C. barbara* or *Tib. quadrisignata*), small species, by contrast, which usually produce softer sounds, and exhibit short flights from one calling site to another, cover a larger range that way (e.g. *Tett. josei* and *Tymp. gastrica*).

2) Species specificity of the sound signals of the cicadas is mainly obtained by the time and amplitude patterns of the songs. The use of different frequency ranges may also play a role (Huber et. al. 1992).

Several elements composing the sound signals of cicadas are involved in order to generate the species-specific characteristics of the songs. They can also be varied within a species in order to produce different signals in different behavioural contexts:

a) First of all, the buckling of the timbals may generate in each timbal cycle different patterns, i.e. number of pulses, and time intervals, in addition to their frequency contents (treated above). The timbals of different species can show a different number of ribs which may buckle in a sequence generating a different number of sound pulses, as in *Tib. quadrisignata*, or they may show even a timbal cycle with only one IN and one OUT pulse as in *Tettigetta* spp. Moreover, the relative amplitude and the frequency of these pulses may vary among the species.

b) Secondly, the timbal muscles are driven by the central nervous system, which may generate several different time patterns using even the same basic timbal cycle (e.g. *Tettigetta* spp.). Moreover, the two timbals can be buckled in different phases, from synchrony as in *Tett. josei* to full antiphase as in *C. barbara lusitanica*. This feature adds more possibilities for

variability, sometimes even within a single species (e.g. *Tib. quadrisignata* show different intertimbal phases in calling song and alarm signal).

c) The timbal muscles do not appear to be able to modify the amplitude of the sound pulses. However, the cicadas have the possibility of further adding variability by using other structures and muscles to modify the basic timbal sound. These include the use of the tensor muscle that can modify the mechanics of the timbal in different ways among different species, as indicated above, and thus to make a fine control on the sound generated by the much more powerful timbal muscles. Another different possibility to modulate the sound pulses is with abdominal movements as in *Tib. quadrisignata* (extension vs retraction; up and down movements). The contraction of the tensor muscle and abdominal muscles, also under control of the nervous system, may be varied in different parts of a song (e.g. courtship or calling song) or among sound signals generated in different contexts (e.g. calling song and alarm signal). The contraction of the tensor muscle may be purely tonic or phasic-tonic dependent on the species. A phasic contraction will allow faster modulations within a song than a tonic contraction of the tensor muscle.

Thus, the species specificity of the cicada sound signals arises from the interplay of the mechanical properties of the sound producing structures (morphology) with the possibilities of their control and modulation by the central nervous system. Adaptive radiation in cicadas contributed to the diversity found in the basic timbal mechanism of cicadas, but a similar morphology and a correspondent similar timbal cycle were kept in phylogenetically related species belonging to the same genus (e.g. Tettigetta spp. or Cicada spp.). However, their songs are very distinct from one another. Evolution of sound production in cicadas has induced a large variety of sound producing structures and mechanisms (see foreword). It is even more impressive to see how the principal mechanism of timballing was differently adapted on several levels, -- i.e. the neuronal level generating the specific motor pattern, the muscular and mechanical level generating the time-amplitude pattern, and the biophysical level determining the radiation, -- in order to fulfill the manifold requirements of the sender in cicada acoustical communication -- the male cicada, which in the other end is submitted to the physical constraints.

Sound reception

1. Foreword

Scientific interests on hearing capabilities of insects date from the beginning of the century (e.g. Forel 1908). Although the first findings were achieved with insects producing sound signals in intraspecific communication contexts, they have since been expanded to numerous other species, and many of them are not known to generate acoustic signals (e.g. lacewings, many moths, praying mantis). It seems that insects possessing hearing structures is a rule and not an exception. Recently, complex tympanic organs were found in tiger beetles (Cicindellidae: Spangler 1988), some flies (Tachinidae: Robert and Hoy 1992; Lakes-Harlan and Heller 1992) and in praying mantis (Mantidae: Yager and Hoy 1986).

A sense of hearing is widespread in the animal kingdom and it has evolved many times independently during the evolution of insects (Michelsen and Larsen 1985). Hearing -- brought to functionality in insects and preserved through natural selection -- incorporates many different constructions (Autrum 1975; Hutchings and Lewis 1983; Michelsen and Larsen 1985) and explores several physical principles (Bennet-Clark 1971; Michelsen and Nocke 1974; Michelsen 1983; Ewing 1989). This wide variety in hearing structures is only conceivable if the ability to hear brought large advantages to animals developing this way of sensing their physical environment.

The prime adaptive functions of hearing are considered to be: a) predator detection and avoidance, and b) intraspecific communication over some distance leading to species isolation. The selective forces for each of those two functions, however, are not necessarily driving the auditory system in the same direction. According to (b), in species using sound communication as the basis for mate recognition, as the majority of cicadas do, an adaptation of the auditory system to the conspecific sounds should have occurred (Popov 1990b). However, if the best hearing range and the spectra of the sound signals are unmatched, which is the case in many cicadas (Popov 1981, 1990b; Popov et. al. 1985; Popov and Sergeieva 1987; Huber et. al. 1990; my own observations in several species), it is likely that other functions such as (a) predator detection and avoidance (Popov 1990b), constitute another important adaptive constraint.

The receiver of a signal in an acoustic communication system has to solve three tasks: 1) to detect the sound, 2) to locate the source, and 3) to filter and recognise its message.

1) The detection of sound sources requests sensitive auditory organs. Moreover, tuning of the hearing system to certain frequencies well represented in a sound signal will filter out these frequencies from the acoustic environment, improving the signal-to-noise ratio and thus allowing a better detection. Sensitive hearing, as found in cicadas, may allow for the detection of mates at a distance, but is also often used to avoid predators (e.g. moths: Roeder and Treat 1961, Roeder 1967; Lacewings: Miller 1984; locusts: Robert 1989; crickets: Popov and Shuvalov 1977, Moiseff et. al. 1978, Nolen and Hoy 1986). Moreover, the signal of the sender and the hearing capabilities of the receiver have to match in order to fulfill these tasks, i.e. detection and recognition of predators and/or matching for conspecific recognition.

2) An accurate localization of the sound emitter is dependent on a good hearing directionality at least in part of the frequency range contained in the signal. The cues for localization of a low frequency sound source by small animals with tympanal organs, which have no intrinsic directional properties, are reduced. They cannot generate a significant sound shadow at low frequencies because of their small body size relative to the wavelength, and the time difference between the excitation of both ears is also very small and unlikely to be detectable by insects (Michelsen and Larsen 1985). Many rely on a pressure difference receiver for directional hearing (Autrum 1940; Michelsen 1983). In this mechanism the sound waves are able to reach both sides of the tympanic membrane, thus allowing a determination of sound direction especially at low frequencies, where the wavelength of the sound wave is large compared with the body size, and scattering effects (shadow) are small. In cicadas the directionality exhibited by the ears is often prominent, even at low frequencies, in spite of the position of the large tympana at the same plane and very close to each other. Although hearing directionality was physiologically demonstrated in some cicadas (Young and Hill 1977; Popov and Sergeieva 1987; Popov 1989,1990a), and extended by the present work to several other species, behavioural tests to their capabilities and performance are largely missing (Doolan and Young 1989).

3) To filter and recognize the message encoded in a sound signal is the ultimate role of hearing. Therefore the animal must be able to differentiate between sounds made by predators,

conspecific signals, and other environmental sounds (e.g. heterospecific or abiotic signals). Although the constraints to achieve such a goal start at the periphery (biophysical properties of the acoustic receiver and capabilities of the primary receptors), this function is only achieved by information processing through the animal's nervous system. Acoustic signal processing by the nervous system in cicadas has received comparatively little attention (Huber et. al. 1980; Huber et. al. 1990), although it may prove to be a very fruitful area of research considering the large variation of signals produced by the cicada males.

An understanding of how these tasks are accomplished can only come from a multilevel analysis, i.e. behavioural observations, morphological and biophysical descriptions of the peripheral mechanisms, auditory nerve recordings, and the investigation of central nervous activity at the single cell level. Furthermore, a comparative analysis of several species might result in insights as to the forces and constraints shaping the auditory performance and capabilities of cicadas.

The following chapters investigate the above mentioned aspects of hearing and are structured according to their approach or method which allows an easier comparison of the species investigated in this thesis at a given level of analysis. Therefore the following chapters will provide: a) a morphological description of the cicada ear (chapter 2), b) a biophysical investigation on to the vibration properties of the tympanum and the acoustic input structures (chapter 3), c) a description of the neuroanatomy of the auditory pathway (chapter 4), d) a description of the information carried to the nervous system by the receptors as studied by auditory nerve recordings (chapter 5), and e) a description of the central nervous processing of this peripheral information (chapter 6). Particular attention to the following aspects will be paid in each chapter: 1) Sensitivity and frequency tuning of the cicada ear, and matching of sender and receiver; 2) Mechanism, inputs, and frequencies for directional hearing; 3) Abilities for frequency discrimination; 4) Copying fidelity of the auditory organ and auditory pathway; and 5) Differences and similarities in males and females. Finally, as the sound producing apparatus of the males, which the females lack, is also

part of the male cicada ear, particular sex specific differences may occur in the study of the auditory system of cicadas.

2. Morphology of the hearing structures

Introduction. Cicadas have a highly specialised auditory system (Fig. 70). As a first step in investigating the auditory structures and the auditory pathway it is necessary to describe the morphology comparatively for the species studied here. The basic structure of the auditory system is similar in all species studied so far (Vogel 1923; Myers 1928; Pringle 1954, 1957; Michel 1975; Young and Hill 1977; Doolan and Young, 1981). The ears are situated

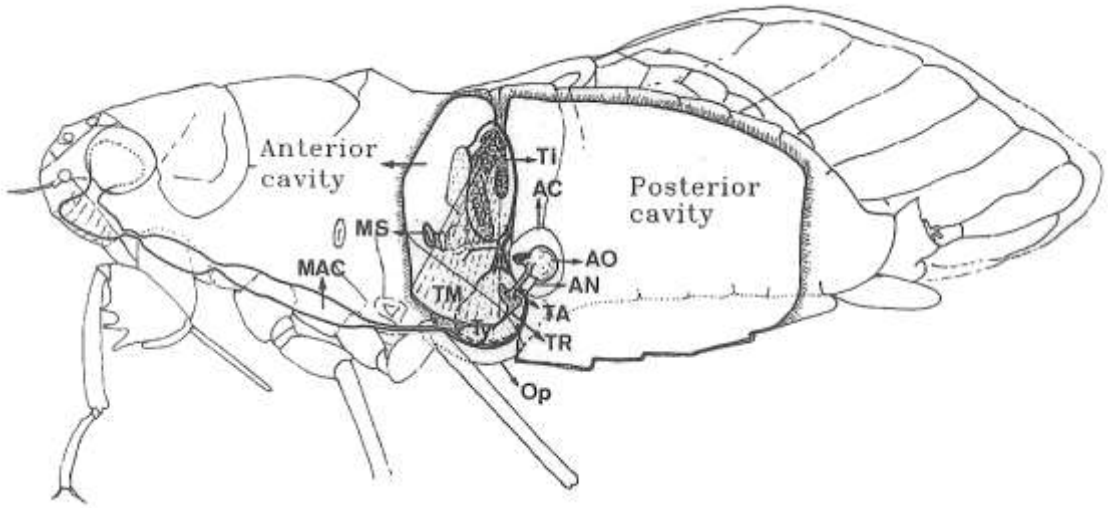


Figure 70 - Diagram of a male cicada showing the auditory system and other structures that may influence hearing. AC Auditory capsule; AN Auditory nerve; AO Auditory capsule; MAC Metathoracic-abdominal ganglionic complex; MS Metathoracic spiracle; Op Operculum; TA Tympanic apodeme; Ti Timbal; TM Timbal muscle; TR Tympanic ridge; Ty Tympanum.

ventrally close to each other in the first abdominal segment. The large tympanic membranes are usually protected by two backward directed projections of the integument (the opercula) arising from the metathoracic epimeron. An extratympanic cavity lies between operculum and tympanum. The volume of this cavity and the gap between opercula and abdomen may be varied by the abdominal movements. The tympana are backed by the air sacs, which are usually much bigger in males, where they occupy most of the abdomen. The tracheal cavities of the males may join together in some species making one large single air chamber. In the female there are

frequently two much smaller chambers divided by a thin longitudinal membrane which usually possesses a foramen connecting both compartments. The chamber(s) provide an acoustical connection between the tympana. The abdominal posterior cavity is connected to the smaller anterior part of the air sac through a passage which in the males lies between the large timbal muscles. This chamber is connected to the outside through the metathoracic spiracles that can be opened and closed by the animal, thus connecting the inner surface of the tympana with the exterior. The folded membranes line the wall of this anterior cavity which, in the males, backs the timbals as well. The tension of the tympanum may be varied by the action of muscles -- the detensor tympani. The tympanic membrane, which is not homogeneous in all its surface, is in many cases very thin and transparent and is usually surrounded by a strong rim, although it may sometimes be relatively slender. The tracheal wall of the abdominal air sac is usually very tightly juxtaposed to the tympanum. On the tympanic membrane there is a thicker sclerotized sclerite widely variable in size and shape -- the tympanic ridge, which connects through a lever -- the tympanic apodeme, to the chordotonal auditory organ. This organ stays inside a strong sclerotized box -- the auditory capsule, situated laterally in the first abdominal segment, and is connected by a ligament to an invagination of tegument -- the attachment horn (Vogel, 1923). Among insects the auditory organs of cicadas are known by the extraordinary number of auditory receptor cells they contain. The auditory nerve arising from the auditory organ travels around the tympanum ventrally and joins several other fibers prior to entering the metathoracic-abdominal ganglionic complex (MAC). The morphology of the tympanic membranes was observed in males and females of six species: *Cicada barbara lusitanica*, *Cicada orni*, *Tettigetta argentata/atra*, *Tettigetta josei*, *Tibicina quadrisignata* and *Tympanistalna gastrica* (Fig. 71).

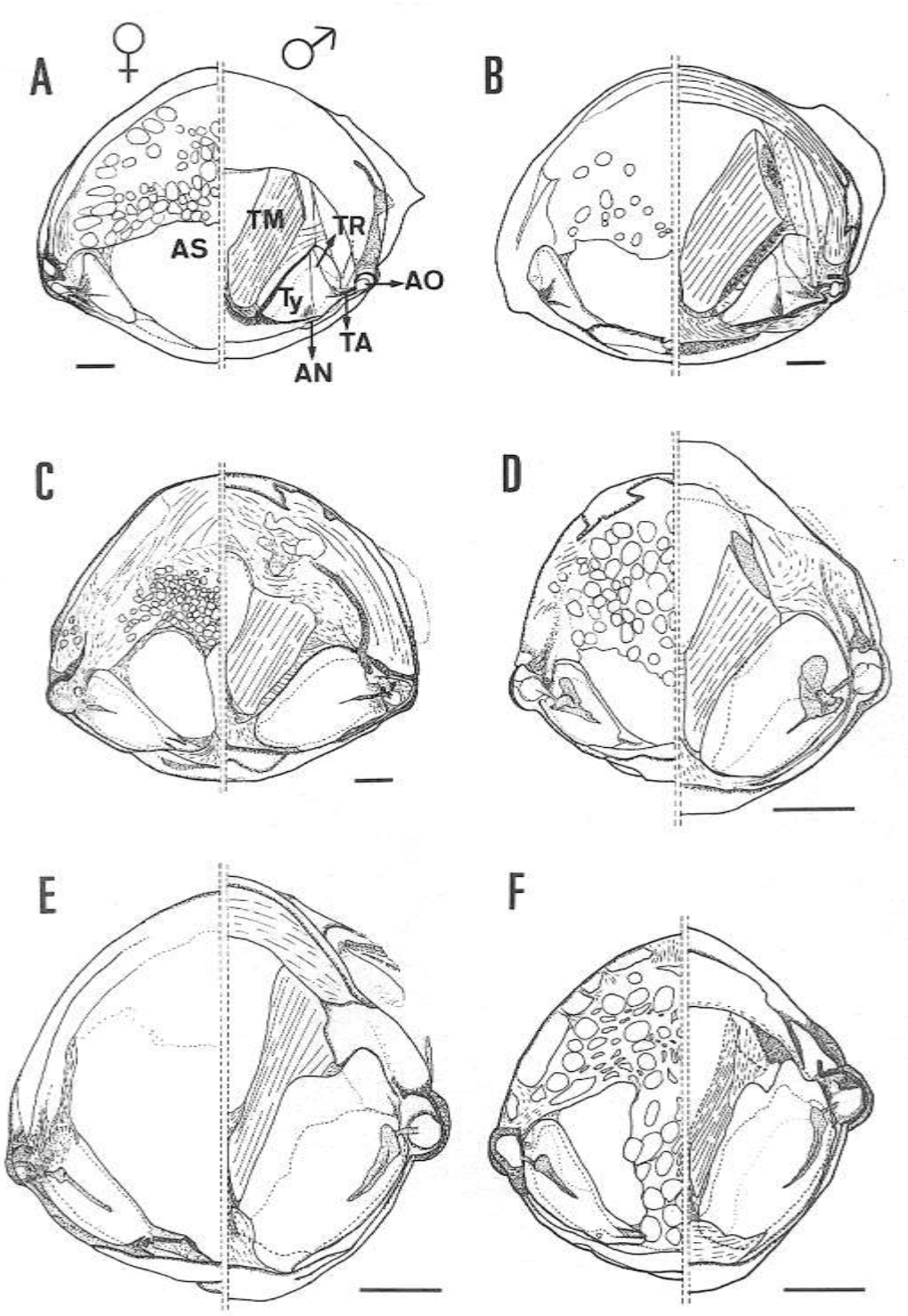


Figure 71 - Morphology of male and female cicada ears as revealed by a transverse cut of the second abdominal segment posterior to the tympana. A) *Cicada barbara lusitanica*. B) *Cicada orni*. C) *Tibicina quadrisignata*. D) *Tympanistalna gastrica*. E) *Tettigetta argentata/atra*. F) *Tettigetta josei*. AS Air sac; AO Auditory organ; AN Auditory nerve; TA Tympanic apodeme; TM Timbal muscle; TR Tympanic ridge; Ty Tympanum. Scale: 1 mm.

In this chapter particular attention is paid to the sex-specific differences, some of which may be attributable to the close association of the sound producing appartatus with the hearing structures in males.

Results. In all species studied the males have larger tympana than females (Table 6). However this relationship varies from less then 2x in *C. barbara lusitanica*, *C. orni* and *Tib. quadrisignata* to 3x-4x in *Tett. argentata/atra* and *Tymp. gastrica*. The tympana are very thin and transparent in all species but *Cicada* spp.. In this case the tympana are less transparent and the tracheal lining of the air sacs is not so perfectly fused with the tympanic membranes. The tympanic ridge is also very different in *C. barbara lusitanica* and *C.orni* relative to the other species (cf. Fig. 71). While in the males of these species the ridge is large and extends across all the membrane (Fig. 71 A,B), in the remaining species it is just projected to the middle of the membrane and is more or less styliform. The ridge is very small in *Tib. quadrisignata* (Fig. 71 C), larger in *Tettigetta* spp. (Fig. 71 E,F) and it becomes much wider in *Tymp. gastrica* (Fig. 71 D). Again, the tympanic ridge is more or less similar in males and females but for *Cicada* species, where in females the ridge is much smaller and does not seem to extend across the membrane (Fig. 71 A,B).

Table 6 - Surfaces of the male and female tympana of some Portuguese cicadas.

Species	Female (mm2)	Male (mm2)	Male/Female
Cicada barbara lusitânica	1.5	2.6	1.7
Cicada orni	1.8	3.5	1.9
Tettigetta argentata/atra	1.2	3.7	3.2
Tettigetta josei	1.1	2.5	2.3
Tibicina quadrisignata	3.0	5.9	2
Tympanistalna gastrica	0.6	2.4	4

It shall be also noted that the tympanic apodeme is not in the same plane as the tympanic membrane, but making an angle with it.

The abdominal air sacs are fused in males but not in females of *Tett. argentata/atra*, *Tett. josei*, *Tib. quadrisignata* and *Tymp. gastrica*. However they are connected by a foramen. While the cavities in females become smaller with time, due to the development of the eggs, in ageing males it occurs the opposite. The tissues lining the abdomen in males become dryer and thinner with age. The abdominal wall is thick in the males of Tibicina and Tettigetta species and much thinner in *Cicada* spp. and *Tymp. gastrica*, especially the ventral wall.

Discussion. The morphology of male and female cicadas was certainly open to different constraints during evolution. For instance, while males should generate and radiate sound signals efficiently in order to insure attraction of mates and thus their reproductive success, females must produce eggs to guarantee that enough offspring would be produced to overcome the next generation. However, both sexes shall keep a good hearing performance.

In face of the consistent differences observed in the hearing systems of both sexes, including the presence of larger tympana in the males which are backed by much larger air sacs also in the males, one may ask if are there correspondingly differences in the frequency responses of the male and female tympana and in their auditory thresholds (question 1). Moreover, these morphological differences probably reflect the above mentioned constraints. As we have seen in the previous chapter, the large air sac in males plays a role in sound production, working as a resonant cavity and/or allowing a large abdominal surface to function as a sound radiator in species with thin walled abdomen. Similarly, since the tympana of the males are also involved in sound radiation (Young 1990, this thesis), they should be large enough to maximize radiation efficiency. The presence of smaller tympana in the females do not necessarily mean that they are compromising their hearing performance. On the other hand, females need to optimise their egg production, thus reducing the internal air sac, but they shall guarantee the presence of enough air backing the tympana in order to allow the membrane vibrations and to keep hearing sensitivity. The acoustic connection between the air backing the tympana, which is an important feature of a pressure difference receiver, shall be maintained as well in order to allow low frequency directionality probably important in the detection and avoidance of predators and also in mate finding. Since there are differences between both sexes regarding the volume of the air sac and the presence of a septum in females, one may ask if this is reflected in the directional hearing performance (question 2).

Other morphological differences of uncertain significance for the hearing performance include: a) Differences in the thickness of the tympana and its lining by the tracheal air sac (e.g. *Tettigetta* spp., *Tib. quadrisignata* and *Tymp. gastrica* vs *Cicada* spp.). b) The size of the tympanic ridge, extending all the way through and linking both edges of the tympanum only in

males of Cicada spp.; these differences do not seem to compromise, however, the hearing performance of *C. barbara* or *C. orni* (see Figs. 93 and 94, page 224), but they may eventually be important in the production of sound signals with lower frequency components as found in these two species relative to the spectra exhibited by the sound signals of the remaining species. c) The modifications in the abdomen wall of the males and in the volume of the air sacs in the females, occurring with age. d) The angle the tympanic apodeme, connecting the tympanic ridge to the auditory organ, makes with the plane of the tympanic membrane, which may eventually permit an easier transmission of several modes of vibration to the receptor cells and thus allowing a cue for frequency discrimination in the auditory organ.

3. Biophysical aspects of hearing:

. Frequency response of the tympanum,

. Acoustical input to the ears, and

. Directional hearing in *Tympanistalna gastrica*.

Introduction. The morphology of the auditory system showed differences in male and female which may result in sex specific differences in auditory threshold and directional hearing capabilities. The particular problems of a small animal in detecting the direction of a sound source were outlined before. In this chapter various measurements were taken to describe the vibrations of the tympanum in order to determine: a) the frequency response, b) the acoustical inputs and c) the directional performance of the cicada ear (question 3).

Basically the structure of the auditory system, as described above, is similar in all species studied so far (Vogel 1923; Myers 1928; Pringle 1954, 1957; Michel 1975; Young and Hill 1977; Doolan and Young, 1981). Relevant morphological features include the ventral position of the tympana, their acoustical connection through the tracheal chamber(s) backing them, the opercula partially closing the extratympanal cavity, and the third spiracles connecting the inner surface of the tympana with the exterior. In *Tymp. gastrica* the opercula extend behind the tympana and the

ears are, in the male, backed by one abdominal air chamber which occupies most of the abdomen. In the female there are two much smaller chambers divided by a thin longitudinal membrane presenting a foramen connecting both compartments.

The directional hearing of male and female cicadas of the species *Tymp. gastrica* was studied by means of laser vibrometry. This research allowed to elucidate the mechanical response of the ears and to study the inputs to the hearing system as well as to identify the ones that might be responsible for creating directional hearing.

Results. The ears respond to frequencies between 2 kHz and 18-20 kHz. The largest vibrations occurred below the peak of the calling song spectrum (compare Figures 21-5 and 78). However, at 5 khz, a frequency well represented in the spectrum of the large sound pulses (Figs. 61, 65), the tympanic vibrations are already about their maximum.

Tympanic vibrations at a certain frequency were linearly related to the sound pressure up to 85 dB SPL in males and 95 dB SPL in females. Above these levels the amplitude of the tympanic vibrations was not proportional to the sound level at all frequencies. Below 8 kHz the vibration amplitude at a certain sound pressure was about 10 dB larger in males than in females (Fig. 72, compare A with B).

Measurements obtained by turning a loudspeaker around the animals and recording the induced tympanic vibrations showed a clear directionality at low frequencies, at which virtually no sound scattering is produced by the body. Furthermore, a large difference in the tympanic vibration (up to more than 20 dB) between the ipsi- and the contralateral positions of the speaker was found in every male, at 12-14 kHz where the largest spectral components of the species' calling song are found.

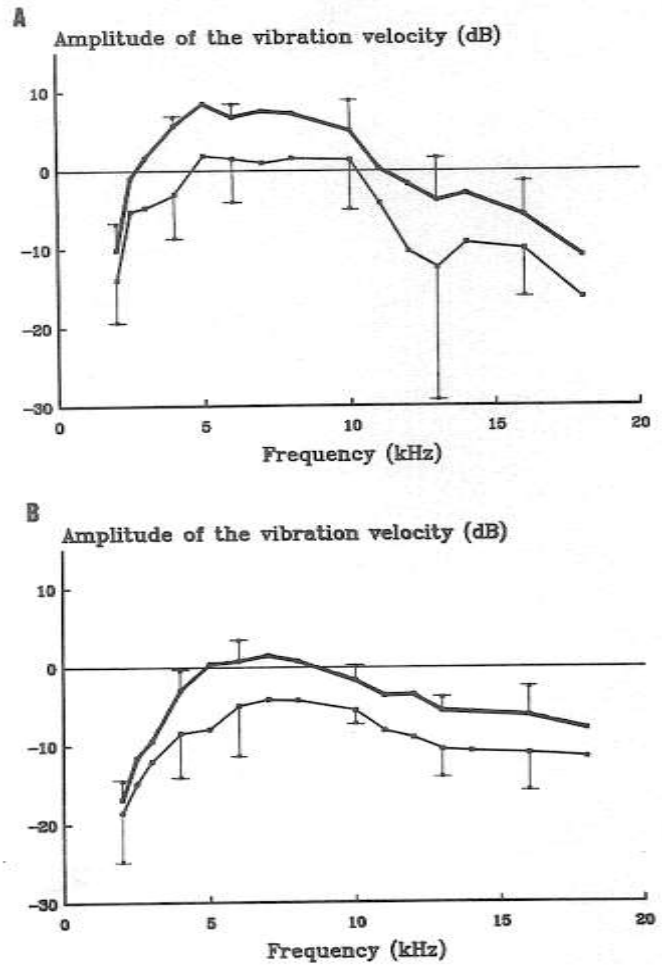


Figure 72 - Frequency response of the tympana of male and female cicadas. The amplitude of the vibration velocity of the tympanic membrane is the average of 21 male (A) and 8 female (B) recordings. The curves are scaled to 80 dB SPL; 0 dB corresponds to 3.2 mm/s. The sound stimulus was presented ipsilateral (thick line) and contralateral (thin line) to the ear. Error bars represent average values plus/minus standard deviation. Calculations were made in linear scale. Note that the logarithmic dB scale makes the upward error bars look smaller than the downward error bars.

A measure of the quality of the sound field during the experiments is shown in Figure 73 A. The homogeneous sound field was made possible by working in an anechoic room, by supporting the animal by means of a very thin and long rod, and by using short sound pulses, which allowed the measurements to end before the distant echoes arrived.

Figure 73 B shows a typical polar diagram of the change in the sound pressure caused by the scattering of the sound by the cicada body. This scattering was similar in males and females which have approximately the same size. Right-to-left differences grow, as expected, with the frequency, but are not more that 4-5 dB at 18 kHz. In contrast, much larger right-to-left differences were seen when the vibration velocity at the same frequencies was plotted as a function of the position of the speaker (Fig. 74). Here the recorded differences were, at low frequencies and in both sexes, from 5 to 10 dB with peaks that could reach 20 dB (see the diagrams for the male at 7.5 kHz and for the female at 5.5 kHz, and Figures 75 A and 76 A). Note

that in contrast to the females, the male diagram also shows a deep null at about 13 kHz for contralateral sound.

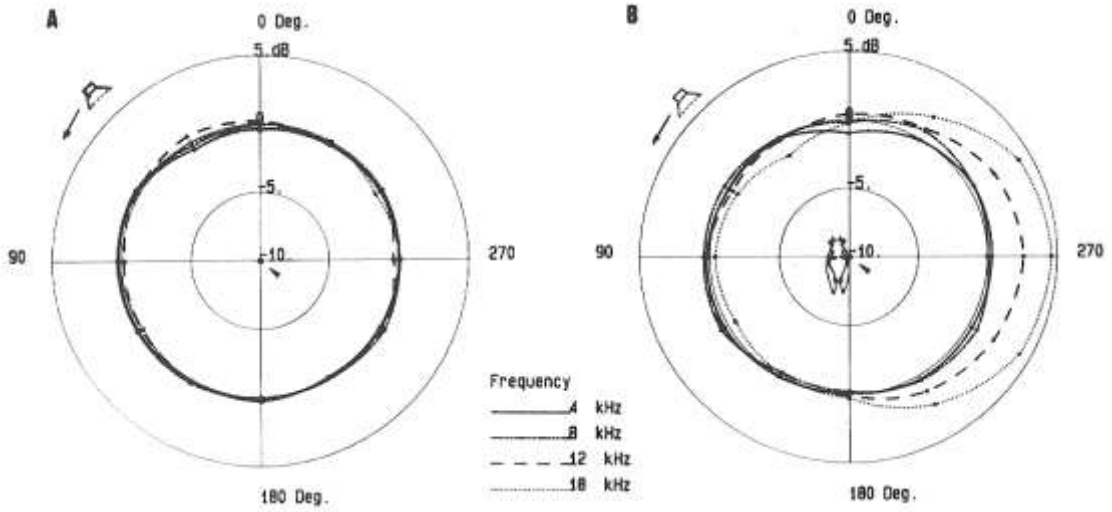


Figure 73 - Scattering of the sound waves produced by the body of a male cicada. A) Control polar diagram of the relative sound pressure at the centre of the roundabout with the holder, but with no animal present. The loudspeaker was turned in steps of 30o. B) As in A, but with the tip of the microphone close to the ear of a cicada. Scattering effects are only observed at 12 kHz and 18 kHz. The arrow indicates the position of the probe microphone at the centre of the roundabout.

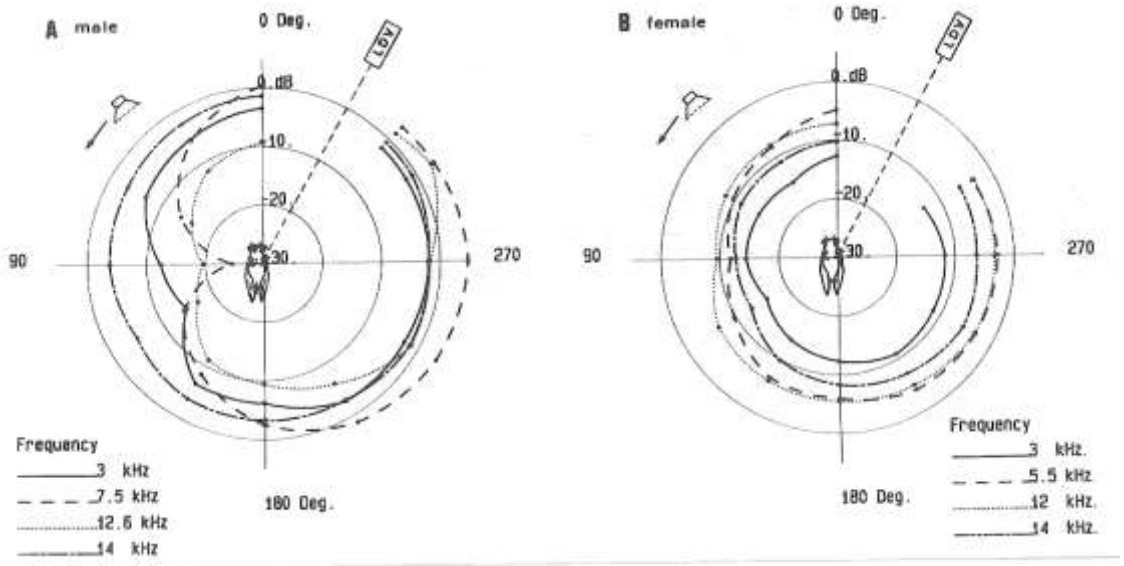


Figure 74 - Directional characteristics of the vibration of a tympanic membrane of a male (A) and of a female (B) cicada at selected frequencies. The sound source was moved around the animal in 30o steps. The curves are scaled to 80 dB SPL; 0 dB corresponds to an amplitude of the vibration velocity of 3.2 mm/s.

In order to identify the mechanisms leading to the strong directionality, several reversible blocking experiments were performed. Typical results are summarized in Fig. 75 (male) and Fig. 76 (female). Figures 75 A and 76 A show the frequency responses of the tympanum in male and female cicadas with sound 0stimulation ipsilateral and contralateral. In the males, the

directionality of the tympanum was high at 3-8 kHz and again around 12-14 kHz. With contralateral stimulation two deep minima were usually found between 3 and 8 kHz and a third minimum between 12 and 14 kHz. These nulls, which were slightly variable

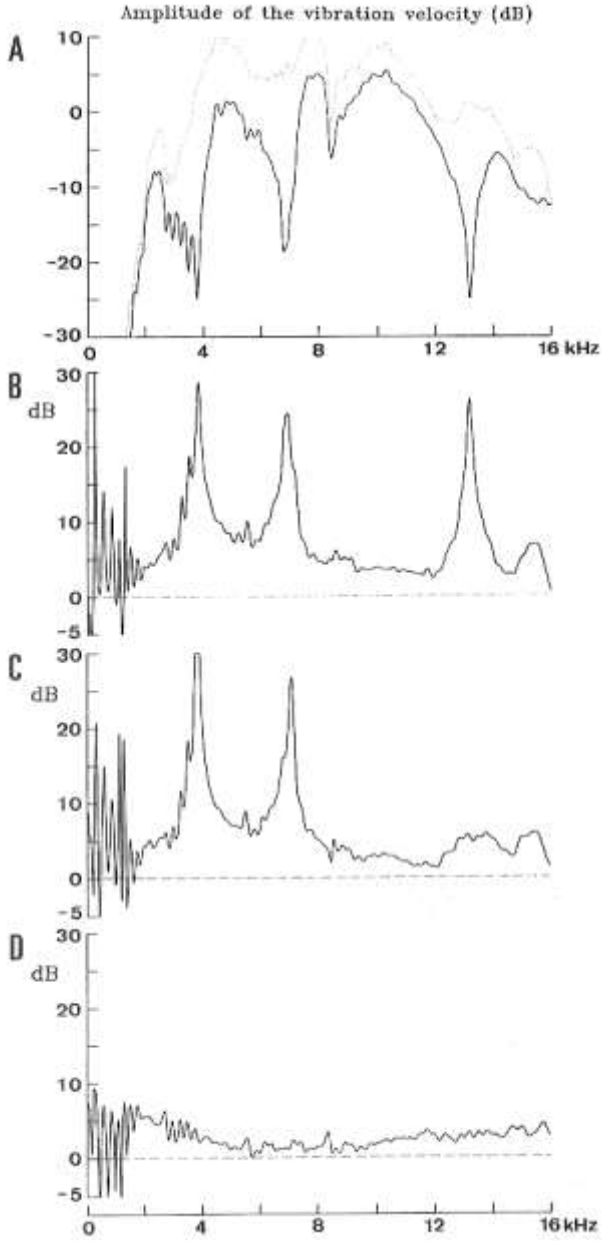


Figure 75 - Effects of blocking the contralateral sound inputs on the directionality of the tympanic membrane of the male cicada. A) Spectra of the tympanic vibrations at ipsilateral sound stimulation (dotted line) and contralateral sound stimulation (solid line). Both opercula had been removed exposing the tympanic membranes. The curves are scaled to 80 dB SPL; 0 dB corresponds to an amplitude of the vibration velocity of 3.2 mm/s. B to D: Directionality expressed as the difference between the vibration velocity at ipsilateral and contralateral sound. B) Initial condition computed from A. C) Bilateral difference after blocking the contralateral timbal. D) The same after blocking the contralateral ear.

in frequency and shape, disappeared (or were strongly reduced) when the speaker was moved to the ipsilateral side. In the females the bilateral difference in the vibrations was present within a larger frequency range, and it was also more regular. The higher directionality was usually found in the range 3-7 kHz. The female did not show the deep null found in the male at 12-14 kHz.

Figures 75 B-D and 76 B-D represent typical effects on the hearing directionality obtained with the blocking experiments. The graphics represent the bilateral difference between the vibration spectrum measured with ipsilateral sound stimulation and that were

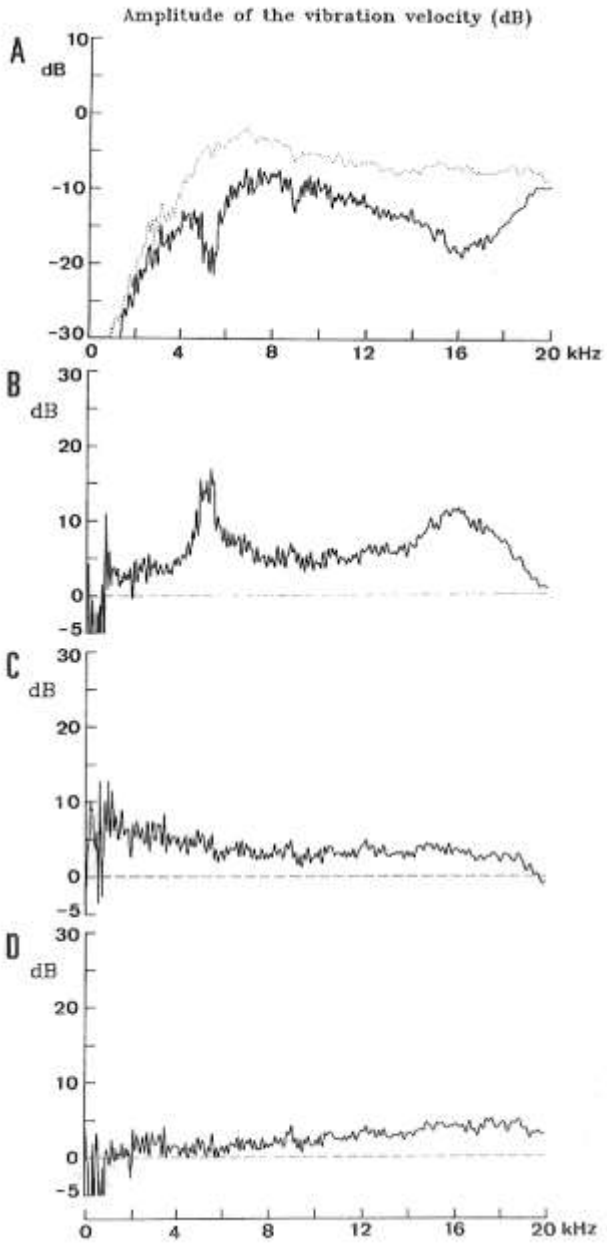


Figure 76 - Effects of blocking the contralateral sound inputs on the directionality of the tympanic membrane of the female cicada. A) Spectra of the tympanic vibrations at ipsilateral sound stimulation (dotted line) and contralateral sound stimulation (solid line). Both opercula had been removed exposing the tympanic membranes. The curves are scaled to 80 dB SPL; 0 dB corresponds to an amplitude of the vibration velocity of 3.2 mm/s. B to D: Directionality expressed as the difference between the vibration velocity at ipsilateral and contralateral sound. B) Initial condition computed from A. C) Bilateral difference after blocking the contralateral ear. D) The same after blocking the contralateral spiracle.

obtained with contralateral sound. Similar experiments were conducted in several cicadas of each sex (eight males and four females), according to different blocking sequences. This experimental procedure was needed in order to avoid systematic errors that might arise in a fixed sequence of blocking experiments, since blocking a sound input not only prevents sound from entering the auditory system, but may also change the acoustics of the system itself.

The general results obtained in these experiments can be summarized as follows: 1) Blocking the contralateral ear leaded to a large reduction of the bilateral differences in the frequency range 2-8 kHz in the male, where the two deep dips found with contralateral stimulation disappeared (compare Fig. 75 C and D), and over 2 kHz in the female (compare Fig. 76 B and C). The differences could be recovered by unblocking the structure. Removing or loading the contralateral tympanic membrane with a small amount of vaseline greatly reduced the directional hearing in the same frequency range. 2) Covering the contralateral timbal of the male drastically diminished the directionality at higher frequencies (12-14 kHz). The null found with contralateral sound stimulation disappeared (compare Fig. 75 B and C) and there was a decrease in the vibration velocity with ipsilateral sound. This large difference was restored after uncovering the contralateral timbal. Slight differences in the position of this null were observed. The null could also be much reduced by loading the contralateral timbal. 3) In the males, covering the abdomen with a thick layer of vaseline resulted in larger tympanic vibrations at frequencies below 2 kHz, with both ipsi- and contralateral sound stimulation. 4) In the females, covering the ipsilateral spiracle did not have effects, but blocking the contralateral spiracle reduced the directionality by increasing the vibration of the tympanic membrane particularly with contralateral sound stimulation (compare Fig. 76 C and D). This effect was reverted by unblocking the structure. In males, where the system is more complex, manipulation of the animal during the experiments might induce changes in the vibrations of the tympana, for instance caused by changes in the shape (volume) of the abdomen (cf. Fig. 78).

Measurements of the sound pressure level inside the abdomen of male cicadas allowed for a rough estimation of the relative importance of several sound inputs to the abdomen (Fig. 77). The sound pressure level dropped inside the abdomen in the frequency

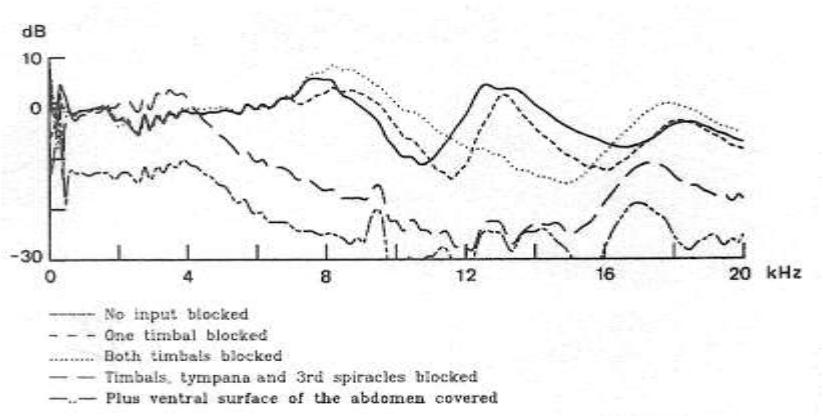


Figure 77 - Effect of blocking the sound inputs to the abdomen, on the inside sound pressure in one male. The speaker was placed in front of the cicada. Zero dB represents no difference between the sound pressure level inside the abdomen relative to the same measurement without the animal.

range 12-15 kHz (by about 15 dB) after blocking the timbals; at this frequency range the peak of the frequency response of the timbal is found (Fig. 68). The block of the tympana and third spiracles markedly reduced the sound input at frequencies higher than 4 kHz, changing by more than 20 dB in the range 8-12 kHz. Covering the ventral surface of the abdomen had a strong effect, especially at low frequencies (10 dB or even more from 0.2-9 Khz).

Experiments changing the shape and volume of the male abdomen showed the importance of this hollow structure for the magnitude of the vibrations of the tympanic membrane (Fig. 78). The shape and size of the abdomen in males could account for differences up to 5 dB (or even more) in the amplitude of the vibrations of the membrane at frequencies from about 2 to 9 kHz, and around 13 kHz.

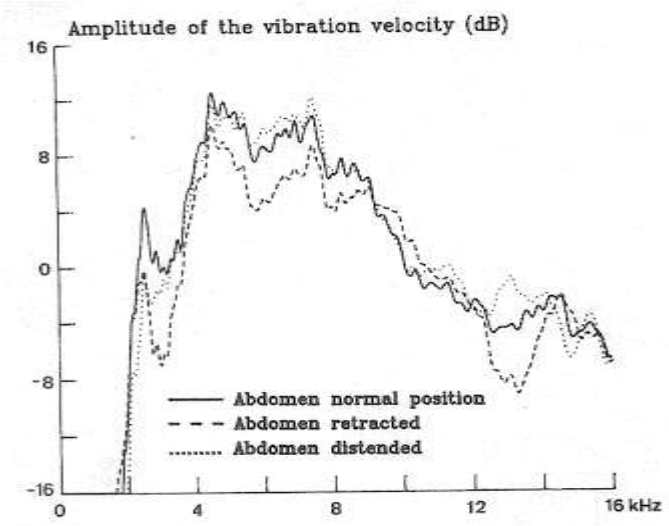


Figure 78 - Effect of the shape (volume) of the abdomen on the response of the tympanic membrane of a male. The amplitude spectrum of the vibration velocity is shown for three positions of the abdomen: normal, retracted and distended. The sound stimulus was presented ipsilateral to the ear. The curves are scaled to 80 dB SPL; 0 dB corresponds to 3.2 mm/s.

Discussion.

Different species of cicadas studied by Young and Hill (1977), Popov and Sergeieva (1987), Popov (1989, 1990a), Fonseca (this thesis) have directional hearing, at least in part of the auditory frequency range. Interaural differences can reach more than 20 dB (Popov 1989,1990a). In some cases, as in Cystosoma saundersii (Young and Hill 1977), Cicadatra spp (Popov 1989) and Cicadetta spp (Popov 1990a), the males lack hearing directionality at low frequencies (below 3 kHz), but this is usually not the case for females. However, the mechanisms responsible for high directionality in cicadas are not understood.

The mechanical response of the ears of the cicada *Tymp. gastrica* was studied by measuring the vibrations of the tympana (comparison with the auditory thresholds derived from whole nerve recordings is made on page 228).

The spectra of the tympanic vibrations does not follow the averaged spectrum of the species' calling song (cf. Figs. 21-5 and 72). Although, if the maximum mechanical sensitivity of the ear is below the peak frequency of the soft sound pulses of the conspecific song (Figs. 61, 65 B), the correspondence is improved comparing the mechanical response of the ear with the spectrum of the large sound pulses (Fig. 61, 65 A). This is an indication that the large sound pulses can better serve distant sound communication in this species.

In the male cicadas the tympanic vibrations may be 10 dB larger than in the females at low frequencies (Fig. 72) (such a difference was also detected in the auditory thresholds, Fig. 98-1). This difference may be related to the presence of larger tympanic membranes and a much bigger abdominal air sac in the males. This view is supported by experiments in which the distension of the abdomen in males was varied. These experiments show a decrease in the mechanical vibrations of the tympanum at low frequencies, and also around 13 kHz, when the abdomen was forced to retract (Fig. 78). Differences in the vibrations of the tympana related to movements of the abdomen were also seen during the LDV measurements with the males. A situation where males were more sensitive than females was reported by Huber et. al. (1990) in *Magicicada cassini*, Popov (1990b) and Popov et. al. (1991) in several other species, whereas the opposite was also found in some large species by Popov (1971) and Popov et. al. (1991), but

not in small ones. In other species both sexes were found to have similar sensitivities at the hearing optima (Young and Hill 1977; Huber et. al. 1990; and my records of auditory nerve thresholds in other species shown in this thesis). In some species females can be much more sensitive than males at high frequencies (my data, for instance in *Tib. quadrisignata* - Fig. 97-1, and Popov, personal communication).

As expected, the scattering of the sound waves by the body of the cicada (Fig. 73 B) increases with the frequency (Michelsen and Nocke 1974). The diffraction starts to be noticeable at relatively high frequencies with wavelengths approaching the size of the animal; at 18 kHz the ipsi- to contralateral difference is no more than 5 dB. The surplus pressure at the ipsilateral side, seen in the measurements above, is similar to the effect observed during scattering of the sound by a sphere (Shaw 1974; see also Michelsen 1983).

Below 8 kHz no scattering effect is seen. Therefore, the measured directionality of the tympanic membrane vibrations at low frequencies is caused by other mechanisms. Figure 74 illustrates typical results in a male and a female cicada. In both cases bilateral differences may be larger than 10 dB (see also Figures 75 A and 76 A).

In order to elucidate the mechanisms and sound input structures responsible for the directionality, several reversible blocking experiments were performed (Figs. 75 and 76). From these data it is obvious that the ears of this cicada are highly complex with several sound inputs which interact to produce bilateral differences in the vibration of the tympana. The ipsi- to contralateral difference at low frequencies in males and females is mainly due to an interaction between the sound waves travelling to the outer surface of the measured ear and to the inner surface via the contralateral tympanum (Fig. 75, compare C and D; Fig. 76, compare B with C). In females the contralateral spiracle is also acting as an important input (Fig. 76, compare C with D). Blocking of the ipsilateral spiracle had no clear effect on the vibrations of the ipsilateral tympanum.

At low frequencies *Tymp. gastrica* thus seems to rely on the mechanism used by the majority of small animals: a pressure-difference tympanic receiver (Autrum 1940; Michelsen 1983). The fact that directionality is greatly reduced by loading or removing the contralateral

tympanum, as well as by blocking, suggests that the contralateral tympanum is changing the sound travelling through it, possibly modifying the phase.

In all the males there also was a deep contralateral null at 12-14 kHz (Fig. 75 A) corresponding to the highest energy components in the calling song. The null disappeared (or was much reduced) when the contralateral timbal was blocked (Fig. 75, compare B and C) or only loaded, but reappeared after the timbal had been unblocked. In males, the timbals are thus another main input to the ears. This conclusion is corroborated by the absence of the null in the timballess females, as well as by the good transmission of the sound to the internal air sac through the timbals at frequencies around 13 kHz (Fig. 77). The blocking of both timbals causes a reduction of nearly 20 dB in the sound pressure inside the abdomen at these frequencies. Somehow the air inside the abdomen and driven by different sources, the timbals and tympana interact in such a way to create the observed directional differences. The mechanism by which interacting sound waves create these large differences in vibration amplitude in this cicada is not understood.

Figure 77 also shows that the ventral surface of the male abdomen is responsible for a large sound input at low frequencies, especially below 4 kHz, a frequency range where the other structures are not very important. This observation is in accordance with the large effect seen in males below 2 kHz when the abdomen is covered with a layer of vaseline.

A directivity pattern compatible with the scattering caused by the body of the animal appeared when the male abdomen was covered with a thick layer of vaseline and after blocking the contralateral ear and the timbal. The same was observed in females after blocking the contralateral spiracle and the contralateral ear.

Cicadas, at least this species, appear to have a highly complex auditory system, which mainly in males may be controlled in several ways, e.g. by changes of the position of the body, the distension of the abdomen, the tension in timbals and tympana or the opening of spiracles. We do not know whether the animals use this possibility for affecting the performance of their auditory system.

One further complication may be caused by the fact that the auditory system changes with the age of the animals. I worked with animals captured in the field, which were of unknown age,

but at least one week old. Differences may arise in the auditory receptor system between recently moulted and older animals. With increasing age the amount of tissue lining the abdominal cavity is much reduced in males, and the air sacs in females become more surrounded by eggs and are reduced in their volume. It is possible that the vibrational properties of the membranes may be affected as well. Also in order to make the measurements with the laser vibrometer it was necessary to remove the operculum covering the ear studied. I do not know how this action affects the vibrations of the ear. Removing the second operculum did not cause any large difference.

4. Neuroanatomy of the auditory pathway

Introduction. Having described the peripheral auditory structures and the biophysical properties of the ear, now the question arises what of the available filtered information is transmitted to the central nervous system. Hence, as a basis for the work on physiological recordings of the auditory nerve (chapter 5) and single cell recordings (chapter 6), here a description of the neuroanatomy of the auditory pathway is given.

Backfills of the auditory nerve (timbal motor axons) were obtained on males belonging to each of the genera studied. This technique was used 1) to confirm the mixed nature of the auditory nerve in these species, 2) to study the projections of the auditory receptor neurons forming the auditory neuropile within the metathoracic-abdominal ganglionic complex (MAC), 3) to investigate the efferent cells sending axons to the nerves, in particular to the separate timbal nerve in *C. barbara lusitanica*, and 4) because the knowledge of the position and shape of the auditory neuropile within the MAC would help to place microelectrodes in order to search for auditory interneurons.

The species observed were *C. barbara lusitanica*, *Tett. argentata/atra*, *Tib. quadrisignata* and *Tymp. gastrica*. The axon fillings were made with nickel from one auditory nerve or simultaneously in both sides with nickel and cobalt (see page 13 for methods). Since the nickel fillings turn blue and the cobalt ones become yellow, it is possible to fill both sides and still interpret in what nerve the cell has its axon. Moreover, since the cells uptaking both nickel and cobalt

become reddish, it is also possible to recognize cell bodies that probably send axons to both auditory nerves.

Furthermore, cross sections of the auditory nerve at several places, observed by electron microscopy (EM), were used to estimate the number of auditory receptor cells present in *C. barbara lusitanica*, *Tib. quadrisignata* and *Tymp. gastrica*, and to determine the sizes of the profiles.

Results. The auditory nerve inserts into the metathoracic-abdominal ganglionic complex (MAC) anteriorly to the abdominal nerves, which insert at the tip of the ganglionic complex. The auditory nerve may insert dorsally relative to the abdominal nerves as in *C. barbara* and *Tett. argentata/atra* or at about the same dorso-ventral level, as seen in *Tymp. gastrica* and *Tib. quadrisignata* (cf. Figs. 82 and 83). The A.N. runs beside the abdominal nerves through the sternal canal and subdivides in a number of branches. Despite the ramifications of the A.N. to the periphery were not studied, in all cases the auditory nerve runs along the ventral rim of the tympanum, innervates the auditory organ, and still subdivides at least twice in *Tymp. gastrica*, *Tib. quadrisignata* and *Tett. argentata/atra* where a branch innervates the timbal muscle, or once in *C. barbara lusitanica*. Before entering the sternal canal the A.N. gives one branch.

Estimations made using electron microscopy cross sections of the male auditory nerve arising from the auditory organ (Figs. 79-81) yielded the following number of auditory receptors:

C. barbara lusitanica --- 1400

Tib. quadrisignata ------ 1270

Tymp. gastrica ---------- 860

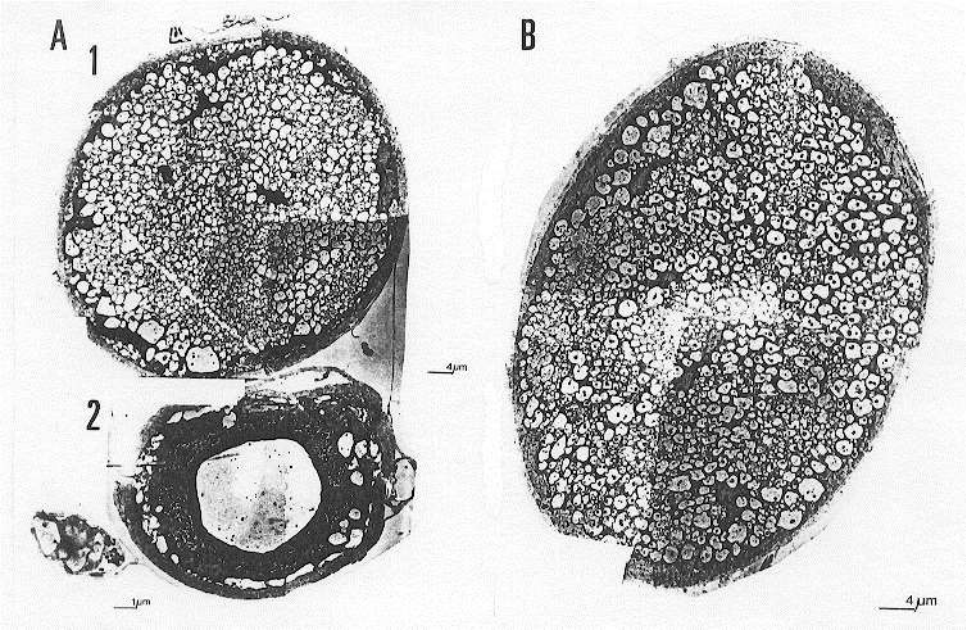


Figure 79 - Electron microscopy observation of transverse sections of the auditory nerve (1) and of the timbal nerve (2) close to the metathoracic-abdominal ganglionic complex (A) in a male of *Cicada barbara lusitanica*. The very large profile on A2 is surrounded by a thick glial layer and corresponds to the big timbal motoneuron. There are about 1400 axon profiles on the nerve arising from the auditory organ (B).

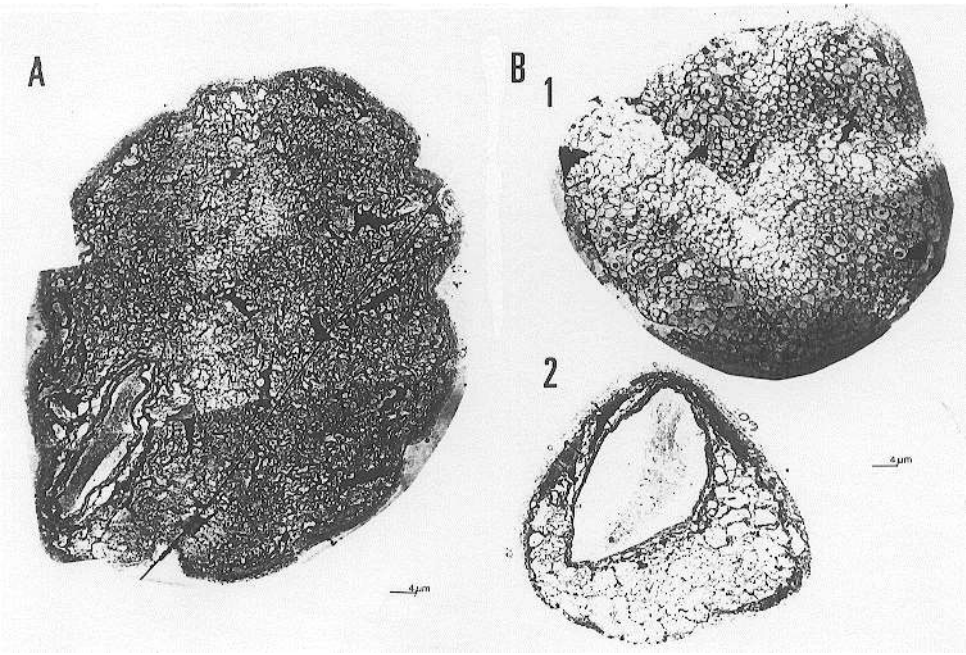


Figure 80 - Electron microscopy observation of transverse sections of the auditory nerve in a male of Tibicina quadrisignata. The sections were obtained close to the metathoracic-abdominal ganglionic complex (A), and after the split to the auditory organ (B). There are about 1270 axon profiles on the nerve arising from the auditory organ (B1). The nerve branch continuing (B2) still includes the very large profile corresponding to the big timbal motoneuron, which is surrounded by a thick glial layer.

The Figures 79-81 show the cross sections of the male auditory nerve after the last split to the auditory organ, the nerve branch continuing and carrying the timbal motoraxons in *Tib. quadrisignata*, and a section made near the ganglionic complex in *Tymp. gastrica*, *Tib. quadrisignata* and *C. barbara lusitanica*. Notice that in *C. barbara* (Fig. 79) the timbal nerve is a

separate one. Apart from the timbal motoneuron, a few other axons run in this nerve but it is not known if they innervate the timbal muscle as well. The very large axon profile corresponds to the timbal motoneuron and it is surrounded by a very thick glial layer (cf. Figs. 79-81).

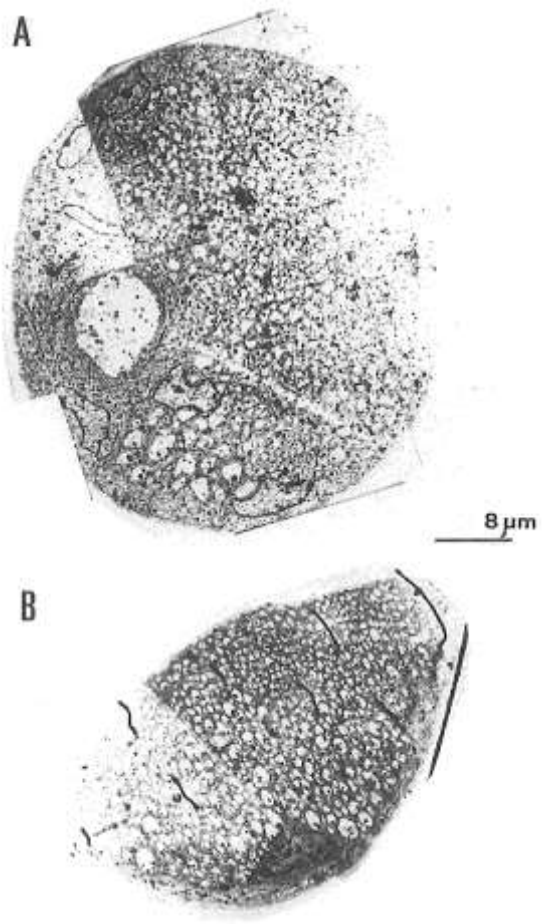


Figure 81 - Electron microscopy observation of transverse sections of the auditory nerve in a male of Tympanistalna gastrica. Section (A) was obtained close to the metathoracic-abdominal ganglionic complex, and includes the very large profile corresponding to the big timbal motoneuron, which is surrounded by a thick glial layer. There are about 860 axon profiles on the nerve arising from the auditory organ (B). It is not shown the nerve branch continuing, which carries the large timbal motoraxon.

The number of axons entering the ganglionic complex was not estimated but it is clearly much higher than the number of profiles exiting from the auditory organ together with the ones integrating the branch to the timbal muscle (Figs. 79, 80), which means that other nerve branches innervating other structures also join the auditory nerve (Vasvary 1966; Young and Hill 1977; Wohlers et. al. 1979). Notice also that the size of the axons exiting the auditory organ is variable, with many small profiles contrasting with some larger ones.

The motor innervation to the timbal muscle runs into the auditory nerve in Tettigetta, Tibicina and Tympanistalna. However, in Cicada this fiber runs in a separate nerve which splits from the auditory nerve soon after it arises from the MAC complex (Figs. 79 A and 82).

In all cases there is a clear metameric organization of the central projections of the sensory receptors that form an auditory neuropile showing bilateral symmetry (Figs. 82 A, 83). In *Tymp. gastrica* the auditory neuropile appears to have nine distinguishable medial projections (Fig. 83-Tg), while in *C. barbara lusitanica* and *Tett. argentata/atra* only eight are clearly seen (Fig. 83-Ta). *Tib. quadrisignata* shows a different intermediate neuropile (Fig. 83-Tq) with only three sharp medial projections in the abdominal region.

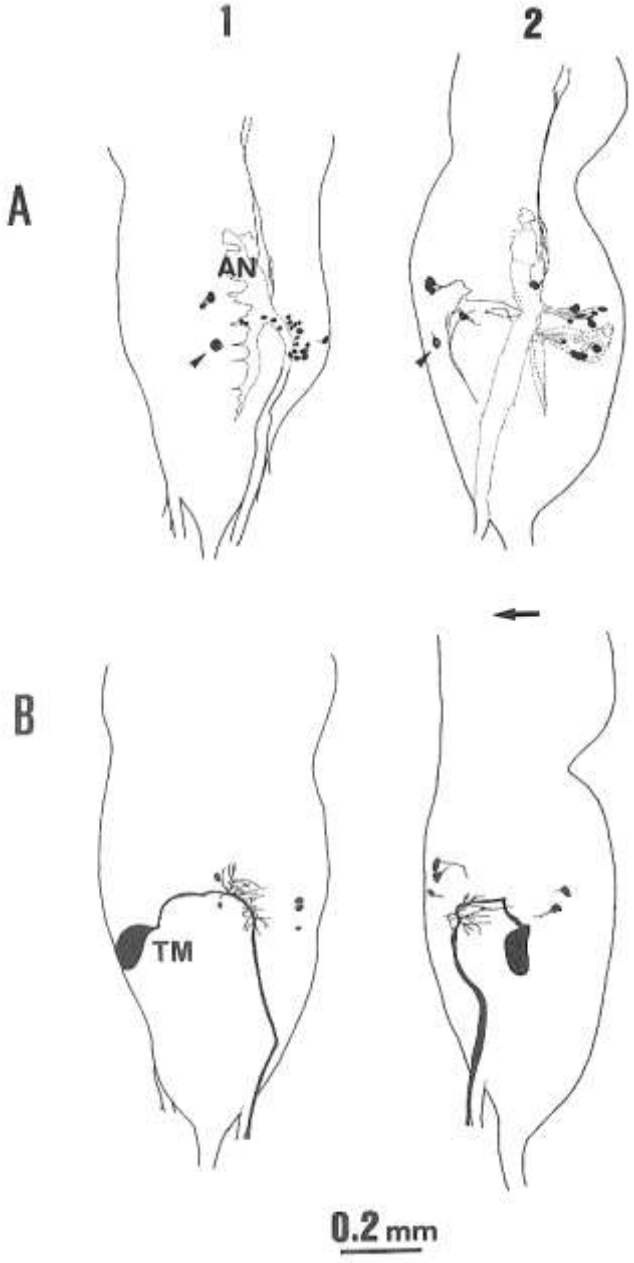


Figure 82 - Axonal fillings of the auditory nerve (A) and of the timbal nerve (B), towards the central nervous system, in a male of Cicada barbara lusitanica. The ganglionic complex was observed from above (1) and in a lateral view (2). The thick arrow indicates the dorsal side. The small arrow points to a cell body appearing reddish in preparations where one auditory nerve was filled with nickel chloride and the other one with cobalt chloride. AN intermediate auditory neuropile; TM Timbal motoneuron.

Nearly all auditory fibers form the intermediate neuropile which extends through the MAC complex. However, in all cases a few fibers project anteriorly along the ipsilateral side in the mesothoracic ganglion (Figs. 82 A, 83) and some of them may reach at least the prothoracic ganglion. In all species some fibers will also project ventrally in the metathoracic and in the anterior abdominal regions (cf. Figs. 82 A2, 83-2).

Many cells with the cell body within the MAC complex send axons to the auditory nerves (cf. Figs. 82, 83). The largest of these efferents is the timbal motor neuron. Its cell body lies medially or slightly dorsally in each side of the abdominal complex. The large axon, which crosses the ganglion, first runs anteriorly shifting then upward and finally runs

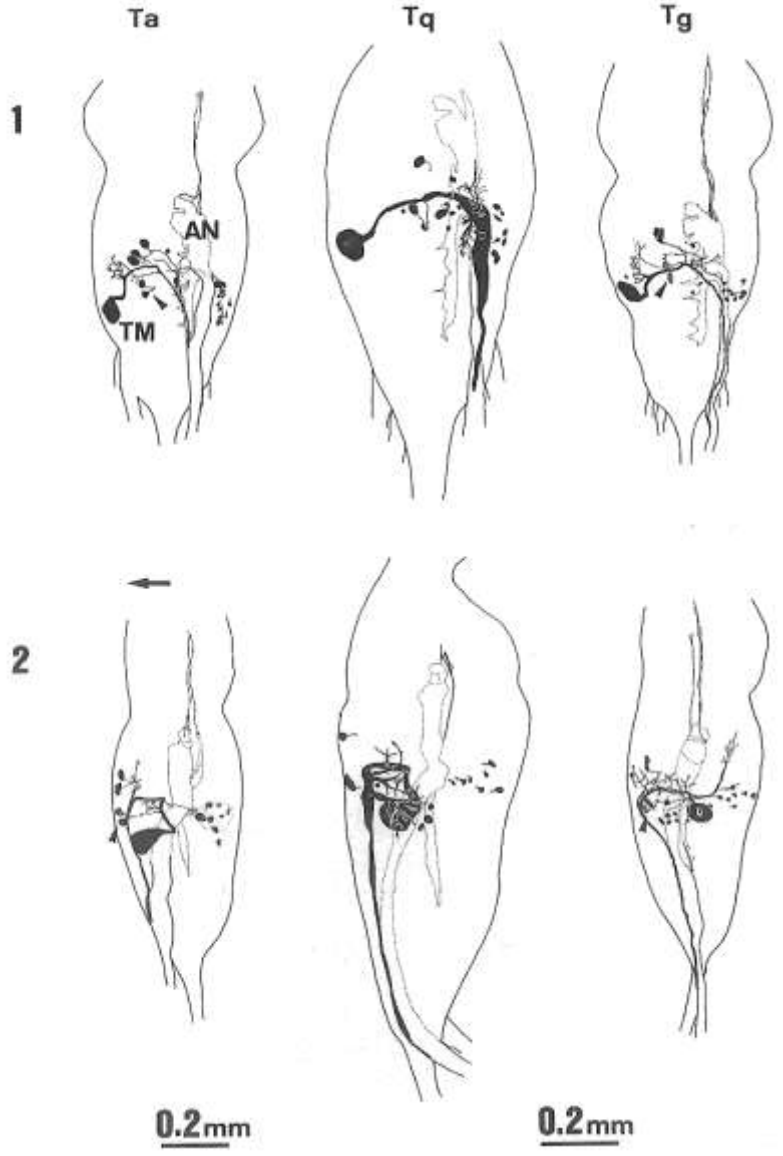


Figure 83 - Axonal fillings of the auditory nerve towards the central nervous system in males of the species Tettigetta argentata/atra (Ta), Tibicina quadrisignata (Tq) and Tympanistalna gastrica (Tg). In these species the timbal motoraxons run in the auditory nerves. The ganglionic complex was observed from above (1) and in a lateral view (2). The thick arrow indicates the dorsal side. The small arrow points to cell bodies appearing reddish in preparations where one auditory nerve was filled with nickel chloride and the other one with cobalt chloride. AN intermediate auditory neuropile; TM Timbal motoneuron.

posteriorly integrating the dorsal region of the contralateral auditory nerve in all cases but *C. barbara lusitanica*. Here the timbal motor axon integrates a separate small nerve (the timbal nerve) which runs posteriorly in a more dorsal position than the auditory and abdominal nerves (Fig. 82 B). In all cases the mirror image pair of neurons come together and cross in the mid line of the ganglionic complex where, at least in some cases (*Tymp. gastrica*, *Tett. argentata*) there is a dendritic region (Fig. 83-2). Another dendritic field, usually the largest, appears in the side contralateral to the cell body and another one may also be present near the cell body. This dendritic field ipsilateral to the cell body was clearly observed in *Tymp. gastrica* and *Tett. argentata/atra* (Fig. 83-2). However it was not noticed in *C. barbara lusitanica* or *Tib. quadrisignata* (Figs. 82 B, 83-Tq 2).

Other efferent neurons also contain axons in the auditory nerve (cf. Figs. 82 and 83). The cell bodies may be ipsilateral or contralateral to the descending axons and they may be situated dorsally or ventrally. Most of these cells have the axons ipsilateral to the cell body and the majority are situated ventrally

Some neurons that seem to be common to the several species can be recognized. From 1 to 3 cell bodies are present in the dorsal medial region of the metathoracic or anterior abdominal ganglia, and they have corresponding mirror image cells in the contralateral side. The descending axons cross the mid line and integrate the contralateral auditory nerve. Another kind of cells have the usually two cell bodies (one cell body in *C. barbara lusitanica*) in the dorsal medial line of the ganglionic complex as well, but they are situated more posteriorly relative to the above mentioned cells. They appear reddish in preparations where the auditory nerves were filled one with nickel and the other with cobalt (Figs. 82 A, 83, arrows). Also common to all species is a group of cells that have their cell bodies located ventro-lateral and the axons course first dorsally and then posteriorly to run on the auditory nerve. Finally, in *C. barbara lusitanica*, there are several efferents running with the timbal motor axon in the timbal nerve. However, I do not know if they are also innervating the timbal muscle.

Discussion. The general anatomy of the CNS revealed by axonal filling was basically similar to the published data (Vasvary 1966; Simmons 1977; Simmons and Young 1978; Wohlers et. al. 1979; Doolan and Young 1981; Popov 1981). Although some species showed some variations.

The auditory nerve is a mixed nerve. The axons of the auditory receptors run together with other sensory afferents and some efferents, namely the motor innervation to the timbal muscle, a condition similar to other described cicadas. Cicada spp. was an exception since here the timbal nerve is a separate one.

The auditory neuropile shows metameric organization, and according to the cited literature the three anterior projections lie in the metathoracic ganglion while the remaining correspond to the fused abdominal ganglia. The number of medial projections was variable among the species. *Tymp. gastrica* showed nine distinguishable medial projections, the same as *Magicicada* spp. (Wohlers et. al. 1979). In *C. barbara lusitanica* and *Tett. argentata/atra* there were only eight clearly seen projections, while *Tib. quadrisignata* exhibited a different intermediate neuropile with only three sharp projections in the abdominal region.

The axons of the large bilateral timbal motor neurons, with the cell bodies in each side of the MAC, cross at the ganglionic mid line where, at least in *Tymp. gastrica* and *Tett. argentata/atra* there is a dendritic region similar to the cases of *Magicicada* spp. (Wohlers et. al. 1979) and *Cicadetta sinuatipennis* (Popov 1981). A different and usually the largest dendritic field is situated contralateral to the cell body, and still another field may be present near the cell body. This dendritic field ipsilateral to the cell body was observed in *Tymp. gastrica* and *Tett. argentata/atra*, and was previously described to other cicadas (Wohlers et. al. 1979; Popov 1981). It was, however, not seen in *C. barbara lusitanica* and *Tib. quadrisignata*, and was also not present in *Cystosoma saundersii* (Simmons 1977).

Wohlers and Bacon (1980) showed that in *Magicicada* the timbal muscle was innervated by several smaller cells, in addition to the large timbal motoneuron. In *C. barbara lusitanica* a few cells run on the separate timbal nerve, apart from the large timbal motor neuron, also well shown in the EM cross sections, but I do not know if they are innervating the timbal muscle as well. Moreover, the profiles corresponding to the large timbal motor neuron are in all species surrounded by a very thick glial layer. The reason for this unusually thick layer is not known but it

may be related with a need for better electrical insulation of this very large axon generating important electrical currents.

The number of auditory receptors in cicadas is the highest known for insects. The estimations presented here agree with the data obtained for other cicadas, which have above 1000 receptor cells (Vogel 1923; Michel 1975; Young and Hill 1977; Wohlers et. al. 1979; Doolan and Young 1981, Huber et. al. 1990), and contrast deeply with the values for other insects using intraspecific acoustic communication (e.g. less than 100 cells in crickets: Ball et. al. 1989, and locusts: Gray 1960; 2 cells in moths: Roeder and Payne 1965). The figure obtained for *C. barbara lusitanica* (1400), is in good agreement with the estimation made by Michel (1975) for the species *C. orni* (1300) belonging to the same genus and having the same size, using cross sections of the auditory organ. *Tymp. gastrica* is a smaller cicada (1.5-1.8 cm long) and has a much smaller number of auditory cell receptors.

The large number of auditory receptors found in cicadas leads to ask why do these insects have so many auditory receptors since the principle tasks may be solved with much fewer cells. The answer is not known as is unknown the mechanism used by cicadas for frequency discrimination. However, since larger cicadas seem to have considerably more receptors than the smaller ones, this is an indication that they may be needed for optimization of the auditory system (functional reasons). Other justifications might include the adaptation of a pre-existing structure composed by so many neurons to a new function -- hearing (phylogenetic reasons).

5. Auditory nerve recordings

Under this chapter I will provide descriptions of the responses of the auditory organ to sound stimulation as well as estimations of the auditory nerve thresholds, based in whole auditory nerve recordings.

5a. Auditory nerve responses

Introduction. The goal of this section was to describe the auditory nerve responses to an external sound stimulus and to self-made sound, centring the attention on a) the synchronization of the auditory receptors, b) on the latency of the auditory nerve response, and c) on copying of the conspecific song by the summed auditory nerve activity. Furthermore, as it was shown before that the auditory nerve contains receptors from other sensory systems (previous chapter), one may also evaluate other responses encoded on the auditory nerve that may be related to other sensory organs with mechanoreceptors (e.g. Young 1975). I also wanted to test if cicadas were able to hear during singing which is important for the detection of predators. This question was raised since the cannon shot experiment made by Fabre (1923), who concluded for the deafness of cicadas during singing. The same was also suggested by Pringle (1954).

Summed responses were recorded on the whole auditory nerve during sound stimulation with pure tone pulses and with the conspecific calling songs in males and females of *C. barbara lusitanica*, *C. orni*, *Tett. argentata/atra*, *Tib. quadrisignata* and *Tymp. gastrica*, but only in males of *Tett. josei*. Moreover, some recordings were also obtained during self generated alarm signal, induced by touching the males of all species but *Tymp. gastrica*. Finally, auditory nerve responses to finger nail clicking used as a sound stimulus were recorded during singing in *Tett. argentata/atra*.

Results. Synchronization of the auditory receptors, i.e. a simultaneous response of many receptor cells, was apparent at the onset of the sound stimuli (conspecific calling song, self produced sound or pure tone sound pulse). The synchronization to the 4 kHz pure tone sound

stimuli, which is within the range of maximum sensitivity in all species (cf. Fig. 92, see also Figs. 93-98), was sharper in the Tettigetta (Figs. 86 A,E, 87 A), species with calling songs characterized by short bursts of sound delivered in short echemes. Usually one or two large peaks were found at the beginning of the response, especially in *Tett. josei*. In contrast, in species with a continuous calling song, as *C. barbara lusitanica*, *C. orni* and *Tib. quadrisignata*, it was common to find up to five synchronized receptor peaks, or even more (Figs. 84 A,E, 85 A,E, 88 A,E). In *Tymp. gastrica* (Fig. 89 A,C) the synchronization was somewhere in between. In all species, the response became strongly synchronized if a broad band very short sound stimulus was presented.

The latencies of the auditory nerve response to an external sound stimulus were around 7-11 ms in the males of all species but the largest, *Tib. quadrisignata*, where the values were somewhat higher (Table 7). Comparing with the males, in females the tendency is for shorter latencies. However only in *Tib. quadrisignata* and *Tymp. gastrica* are the latencies measured in the females clearly shorter than the males' latencies (Table 7).

The auditory nerve latency measured with external sound stimulation was generally similar to the latency observed during the production of sound by the male cicadas before and after cutting the auditory nerve close to the ganglia (cf. Figs. 84 A-D, 85 A-D, 86 A-D, 87 A-D, 88 A-D), which still allowed a response to a motor action of the ipsilateral timbal only in Cicada spp., and after compensation for the speaker distance (approximately 40 cm). However, in many recordings made during singing the nerve activity could be observed with much shorter latencies or even before the sound was produced (e.g. Tettigetta species, Figs. 86 C,D, 87 C,D), which was present even after the nerve was cut close to the ganglia.

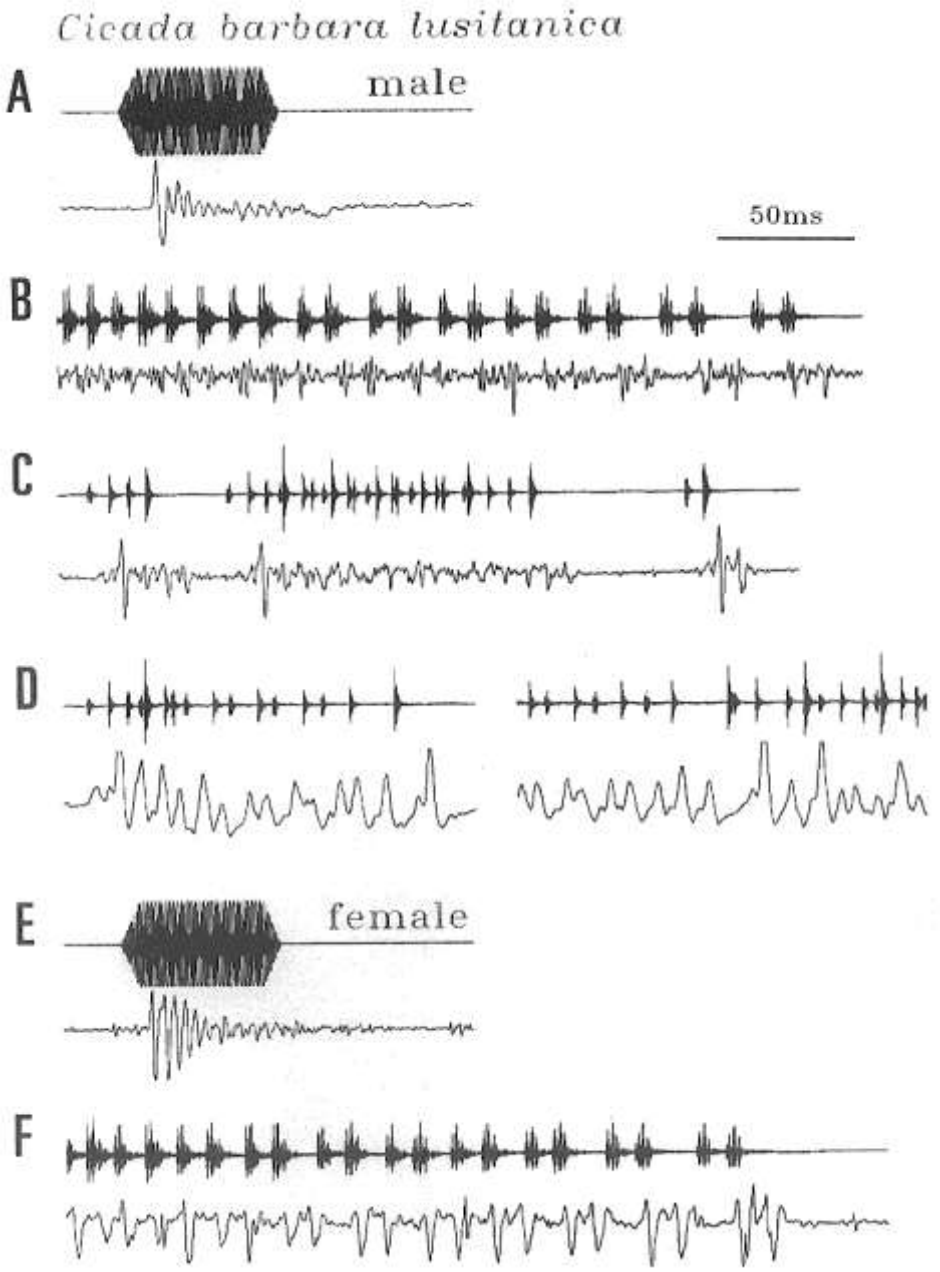


Figure 84 - Summed auditory nerve activity obtained as a response to several sound stimuli on male (A-D) and female (E,F) *Cicada barbara lusitanica*. A,E) Summed response to a pure tone stimulus of 4 kHz, 95 dB SPL, showing the synchronization of the receptors at the onset of the sound stimulus. B,F) Examples of copying of the structure at the end of a song sequence by the nerve signal. C,D) A male auditory nerve activity during self made sound, before (C) and after (D) cutting the auditory nerve close to the ganglionic complex. Timbal motoneuron activity is not seen because in this species there is a separate timbal nerve.

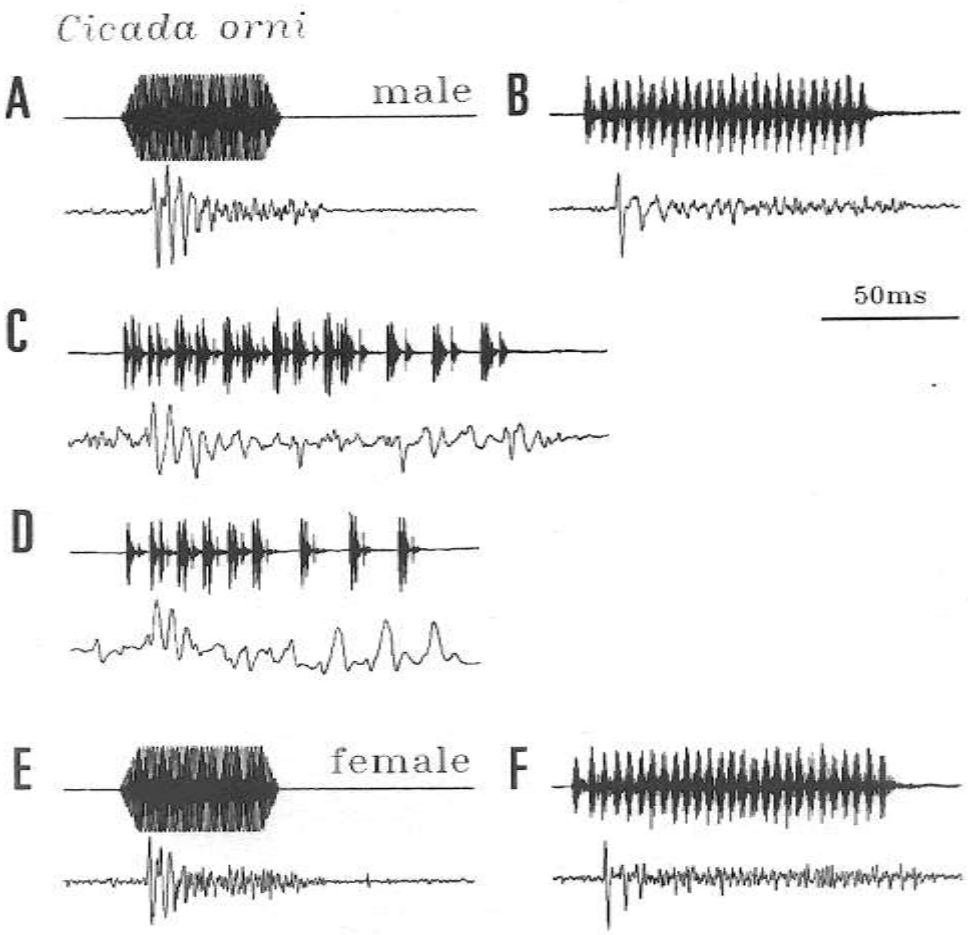


Figure 85 - Summed auditory nerve activity obtained as a response to several sound stimuli on male (A-D) and female (E,F) *Cicada orni*. A,E) Summed response to a pure tone stimulus of 4 kHz, 95 dB SPL, showing the synchronization of the receptors at the onset of the sound stimulus. B,F) Whole nerve response to the play-back of a calling song echeme. C,D) A male auditory nerve activity during self made sound, before (C) and after (D) cutting the auditory nerve close to the ganglionic complex. Timbal motoneuron activity is not seen because in this species there is a separate timbal nerve.

The general structure of the calling song is copied by the whole nerve response in all species (cf. Figs. 84 B,F, 85 B,F, 86 B,F, 87 B, 88 B,F, 89 B,D). However, copying of the individual timbal actions seemed only to occur in species where the repetition rate was not very fast (e.g. *Tib. quadrisignata*, Fig. 88 B,F) or in parts of the song where the rate is slowed down (e.g. *C. barbara lusitanica* at the end of the song sequence, Fig. 84 B,F). The individual sound pulses generated during the calling song are usually repeated at a high frequency and, in spite of being very well reproduced by the tympanic vibrations, at least in *Tymp. gastrica* (Fig. 90), it was not possible to see a strict correlation between them and the auditory nerve recordings (e.g. Fig. 89 B,D).

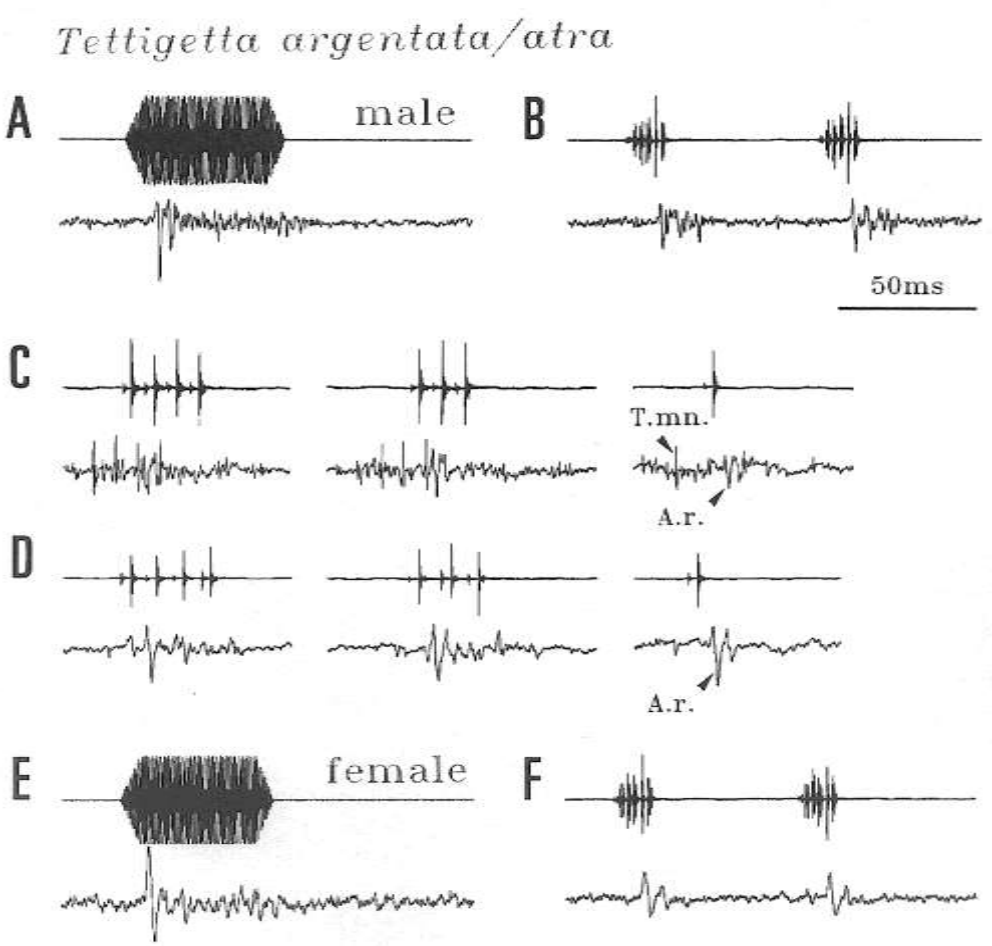


Figure 86 - Summed auditory nerve activity obtained as a response to several sound stimuli on male (A-D) and female (E,F) *Tettigetta argentata/atra*. A,E) Summed response to a pure tone stimulus of 4 kHz, 95 dB SPL, showing the synchronization of the receptors at the onset of the sound stimulus. B,F) Whole nerve response to the play-back of calling song echemes. C,D) A male auditory nerve activity during self made sound, before (C) and after (D) cutting the auditory nerve close to the ganglionic complex (only one timbal is generating sound). The timbal motoneuron activity is suppressed by cutting the nerve. A.r. Auditory response; T.mn. Spike from the big timbal motoneuron.

Table 7 - Latency range of the auditory nerve response to a sound pulse of 4 kHz in some cicadas from the Portuguese fauna.

Species	Male (ms)	Female (ms)
Cicada barbara lusitânica	(5) 8.1-11.4	(4) 7.8-9.1
Cicada orni	(5) 8.1-10.7	(1) 8.1-8.7
Tettigetta argentata/atra	(5) 7.1-9.7	(1) 7.6-7.8
Tettigetta josei	(5) 7.2-8.9	----
Tibicina quadrisignata	(5)11.9-14.6	(4) 7.7-11.0
Tympanistalna gastrica	(5) 7.2-9.9	(4) 5.1-7.6

(Numbers in brackets indicate the number of cicadas measured. The latency was measured from the onset of the sound stimulus, a shaped 4 kHz pulse with 95 dB SPL at the animal level, to the beginning of the first synchronized auditory response. The values are corrected for the speaker-to-animal distance. The response latency to four sound pulses was measured in each animal, and in many cases in both auditory nerves).

In *Tett. argentata/atra* (Fig. 86 C), *Tett. josei* (Fig. 87 C) and *Tib. quadrisignata* (Fig. 88 C), a large spike correlated to the activity of the big timbal motoneuron can be seen in the auditory

nerve response during singing. The spike disappeared after the nerve was cut proximally to the ganglia (Figs. 86-88 D). In *Cicada barbara* (Fig. 84 C) and *C. orni* (Fig. 85 C), this spike was not present in the recordings since the large timbal motoneuron does not run in the auditory nerve, but in a small branch which separates from the auditory nerve near the MAC ganglionic complex (cf. page 202).

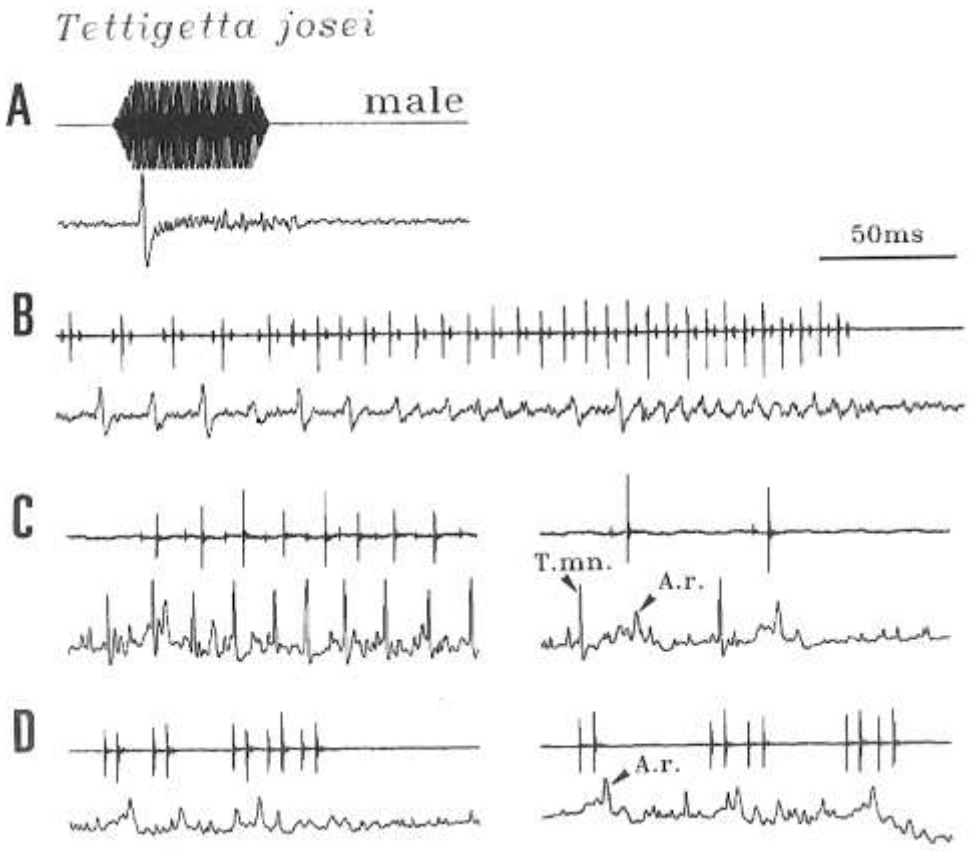


Figure 87 - Summed auditory nerve activity obtained as a response to several sound stimuli on a male of *Tettigetta josei*. A) Summed response to a pure tone stimulus of 4 kHz, 95 dB SPL, showing the synchronization of the receptors at the onset of the sound stimulus. B) Example of copying of the calling song structure by the auditory nerve signal. C,D) A male auditory nerve activity during self made sound, before (C) and after (D) cutting the auditory nerve close to the ganglionic complex. The timbal motoneuron activity is suppressed by cutting the nerve. A.r. Auditory response; T.mn. Spike from the big timbal motoneuron.

Stimulation of the hearing structures of *Tett. argentata/atra* with short broad band sound stimuli generated by finger nail clicking, during singing elicited by brain stimulation, elicited a response in the auditory nerve correlated with the sound stimulus if it was generated between the song echemes (Fig. 91). However, when the stimulus was produced close after the song echeme, there appeared to be a reduced response amplitude of the summed auditory nerve activity.

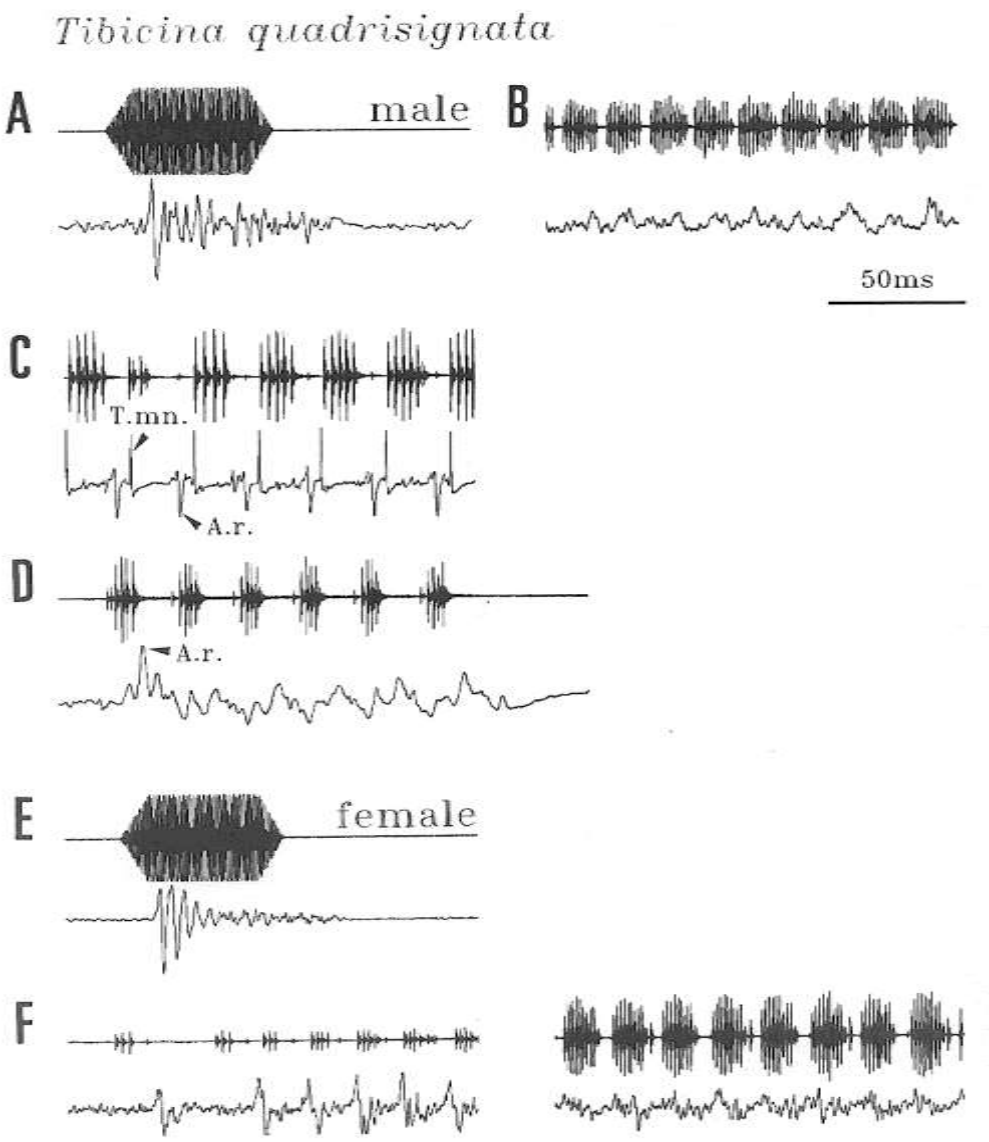


Figure 88 - Summed auditory nerve activity obtained as a response to several sound stimuli on male (A-D) and female (E,F) *Tibicina quadrisignata*. A,E) Summed response to a pure tone stimulus of 4 kHz, 95 dB SPL, showing the synchronization of the receptors at the onset of the sound stimulus. B,F) Example of copying of the calling song structure by the auditory nerve signal. On the female is also shown the response to the play-back of the beginning of a calling song sequence. C,D) A male auditory nerve activity during self made sound, before (C) and after (D) cutting the auditory nerve close to the ganglionic complex (only one timbal is generating sound). The timbal motoneuron activity is suppressed by cutting the nerve. A.r. Auditory response; T.mn. Spike from the big timbal motoneuron.

Discussion. A synchronized auditory nerve activity similar to the observation of the auditory nerve responses of the species studied here was found in other cicadas by Pringle (1954), Enger et. al. (1969) and Huber et. al. (1980). Such synchronised responses were caused by simultaneous stimulation of many receptor neurons and shall be dependent on the mechanics of the ear and on the transmission of the vibrations to the auditory organ through a single tympanic apodeme. In this context, frequency discrimination seems only possible if several modes of vibration of the tympanum could be transmitted to several groups of auditory sensory cells within

the compact auditory organ, a question not understood yet. The number of synchronized receptor peaks found in the summed response, also probably dependent on the mechanics of the auditory system and the properties of the receptor cells, was smaller in species producing calling songs with short echemes relative to the species with continuous songs. This may possibly allow cicadas with songs characterized by short bursts of sound delivered in short echemes to follow the time pattern of their conspecific signals.

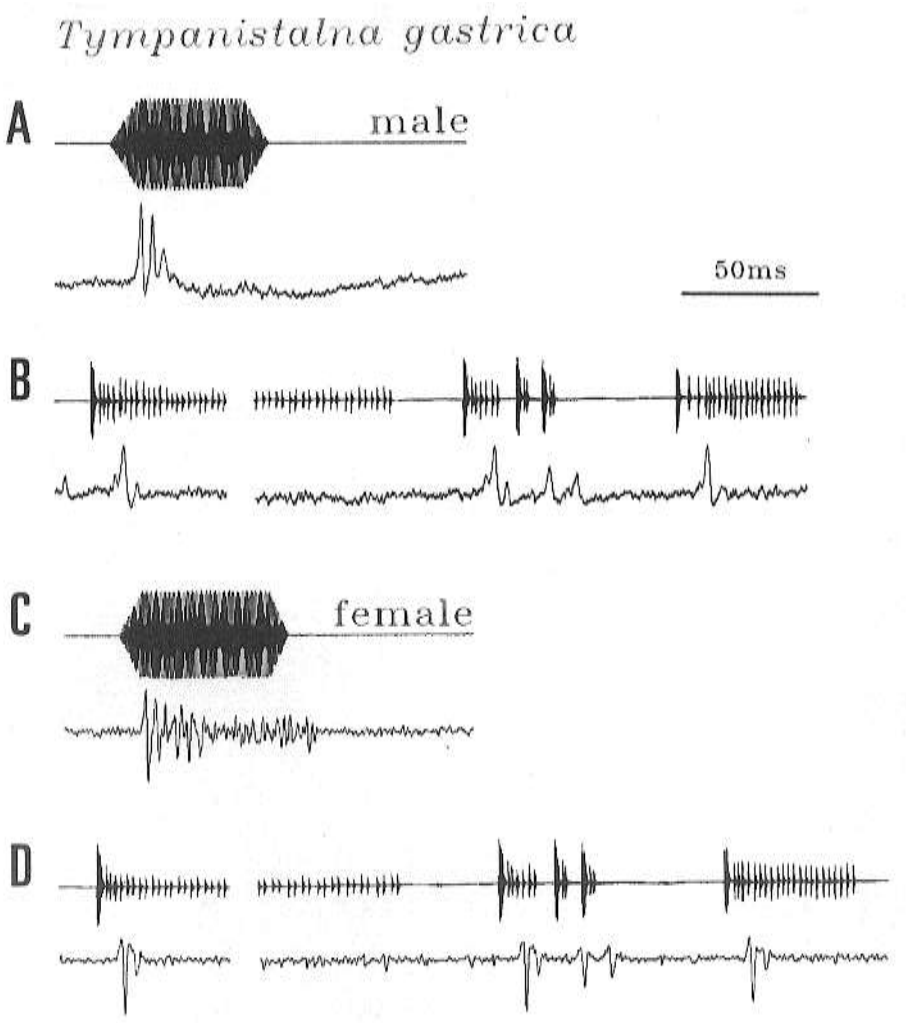


Figure 89 - Summed auditory nerve activity obtained as a response to several sound stimuli on male (A,B) and female (C,D) *Tympanistalna gastrica*. A,C) Summed response to a pure tone stimulus of 4 kHz, 95 dB SPL, showing the synchronization of the receptors at the onset of the sound stimulus. B,D) Auditory nerve signal as a response to the play-back of a calling song sequence with several different echemes.

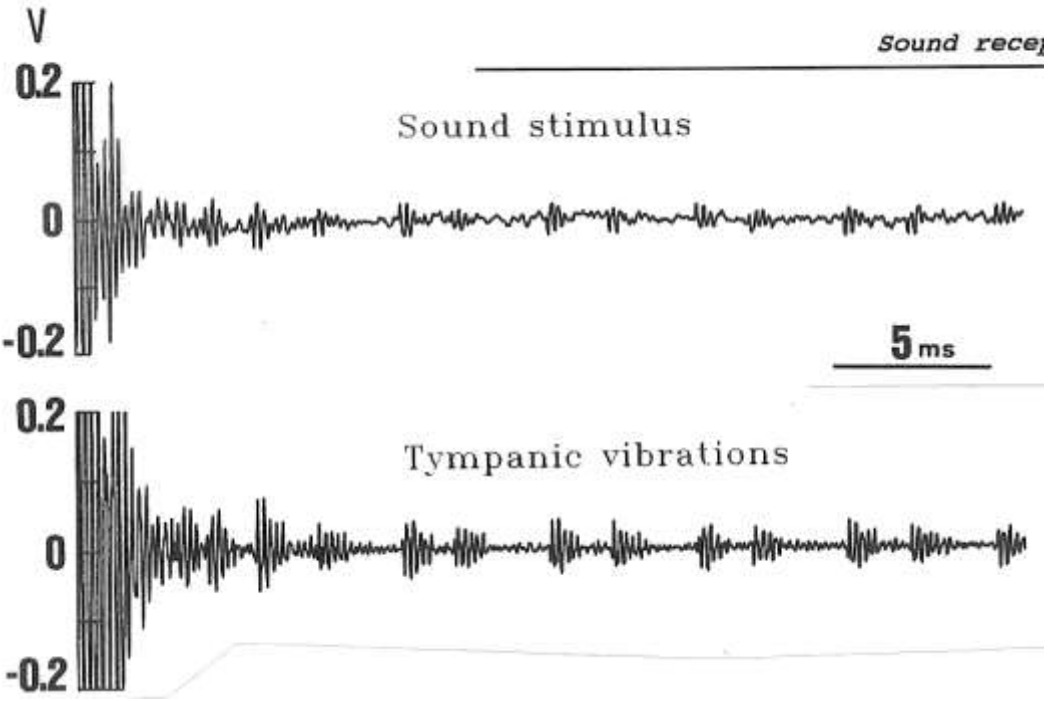


Figure 90 - Tympanic vibrations induced on *Tympanistalna gastrica* by sound stimulation with a calling song echeme. The temporal pattern within the echeme is strictly followed by the vibrations of the tympanic ridge. The laser vibrometer calibration corresponds to 10.4 mm s-1 V-1.

The latencies of the auditory nerve responses to external sound stimulus, between 7 and 11 ms in all species but males of the largest *Tib. quadrisignata* and females of the small *Tymp. gastrica*, were in good agreement with the ones found by Enger et. al. (1969) in the Brazilian species *Fidicina rana* (about 10 ms) and by Huber et. al. (1990) in *Magicicada septendecim* (around 8 ms) when excited by its self generated sound. According to Huber et. al. (1980, 1990), however, in this and other North American species the latencies of the

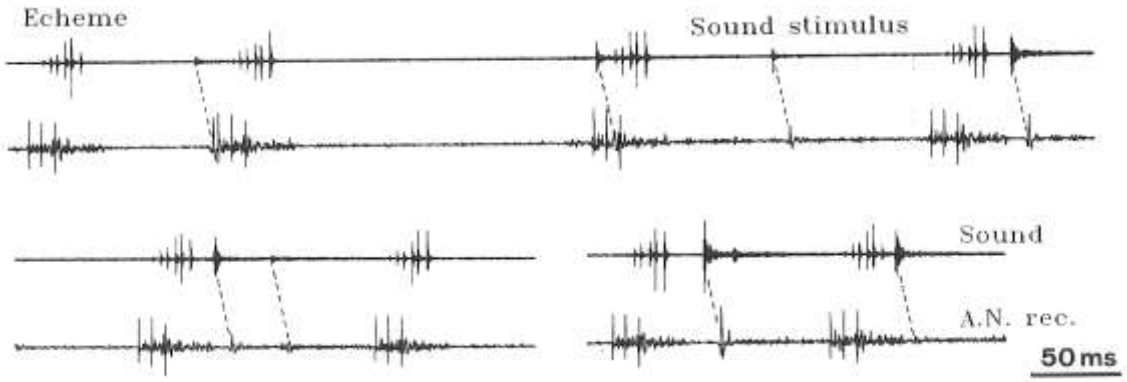


Figure 91 - Summed auditory nerve responses to a sound stimulus presentation (finger nail clicking) during singing by a male of *Tettigetta argentata/atra*, obtained as an after-effect of electrical brain stimulation. The amplitude of the nerve signal is dependent on the position of the sound stimulus relative to the song echeme.

auditory nerve response were much longer (about 20 ms) with an external sound stimulus. Contrasting with the observations of Huber et. al. (1990), here I found the same latencies of the auditory response either to an external acoustic stimulus or to self made sound. I also observed activity in the auditory nerve with much shorter latencies, sometimes even before the sound was produced by the cicada, which indicated the response of other sensory systems (e.g. mechanosensory chordotonal organ, Young 1975). This activity was clear after removing the efferent activity by cutting the auditory nerve close to the ganglia.

The latencies depend on the size of the cicadas and on the emplacement of the hook electrode. Therefore, differences among the species (Table 7) were expected. In smaller species, as well as in females relatively to the males, there is a reduction in the length of the auditory nerve segment from the auditory organ to the place where the measuring electrode was inserted, and thus a shorter conduction time from the transduction point to the recording site is measured. One should be aware that some incertitude in the latencies measured here might arise from the use of a shaped pulse, but this error shall be relatively constant in each species, and also small due to the use of loud stimuli.

In spite of the fact that the nerve responses reflected the general structure of the calling song in all species studied here, copying of the individual timbal actions as shown by Huber et. al. (1980) and Huber (1983) in Magicicada and Okanagana rimosa, seemed only to occur in cicadas with a slower timbal buckling repetition rate, and in parts of the songs where the rate was slowed down (< 60 to 80 Hz).

Tettigetta argentata/atra appeared to be able to hear during the sound pauses between the echemes generated during the calling song in some preliminary and simple experiments, a problem subjected to controversy since the observations by Fabre (1923) with his cannon shot experiment. This is certainly an advantage for the animal, to be able to detect sounds made by predators during singing, or in order to synchronize in a chorous with other singing cicadas. The causes of the auditory response suppression when the sound stimuli came close to the self generated echeme are unknown in this species. Although, they are likely to be caused by the contraction of the detensor tympani muscles -- a process first postulated by Pringle (1954) and shown in recent work by Hennig et. al. (1994b), who demonstrated modulations in the auditory

nerve response induced by tympanic folding generated in soundless singing males of a North American cicada, Tibicen linnei.

5b. Auditory nerve thresholds and hearing

Introduction. In species relying on sound communication for bringing the sexes together and thus allowing mating, as cicadas do, it is often found a high sensitivity of the auditory organ to frequencies well represented in the calling song spectrum (e.g. crickets: Weaver and Vernon 1959, Esch et. al. 1980; some cicadas: Simmons et. al. 1971, Young and Hill 1977, Huber et. al. 1990). In other insects the auditory system may be particularly responsive to sounds associated with predators (e.g. lacewings: Miller 1971, 1982; moths: Roeder 1966, Fullard and Thomas 1981; mantids: Yager and Hoy 1986). In many cicada species (e.g. Popov 1981, 1990b), as well as found in reports from other insects (Mason 1991), the best sensitivity of the ear occurs at frequencies much below the spectral peak of the calling song. These observations suggest an adaptation to another function besides the intraspecific communication. In addition to a high sensitivity, one would also expect good directional hearing, since this would allow a faster localization of the emitter.

The goals of this study were 1) to describe the range of best hearing sensitivity and the roll off of the tuning curve, as well as to compare the auditory nerve threshold curves among the species, 2) to compare the auditory nerve threshold curves with the spectra of the conspecific songs in order to describe the accuracy of matching of sender and receiver in this communication system, and 3) to estimate the directional hearing capabilities of the species, i.e. if the hearing organ uses the available information at the periphery described in chapter 3.

In order to carry out these investigations, auditory nerve thresholds were obtained in six species of cicadas with the headphone monitoring method from 1.5-2 kHz to 40 kHz, using pure tone sound pulses up to 90-95 dB SPL. The sound was presented ipsi- and contralateral to the ear under study. The species studied were *C. barbara lusitanica*, *C. orni*, *Tett. argentata/atra*,

Tett. josei, *Tib. quadrisignata* and *Tymp. gastrica*. Males and females were monitored in all species but *Tett. josei*, where only males were studied.

Results.

1. Auditory nerve thresholds. Males and females of all species showed the lowest thresholds in a narrow frequency range from 3-5 kHz (Fig. 92), despite the different power spectra found among the songs produced by the several species (cf. Figs. 10, 12, 14, 20, 21). The highest sensitivity, around 25-30 dB SPL on average (with one animal presenting a value below 20 dB), was found in *Tib. quadrisignata* (the largest species) (Fig. 97 A1) and *Tett. argentata* (a medium size species) (Fig. 95 A1). On the opposite, *Tymp. gastrica*, one of the smallest

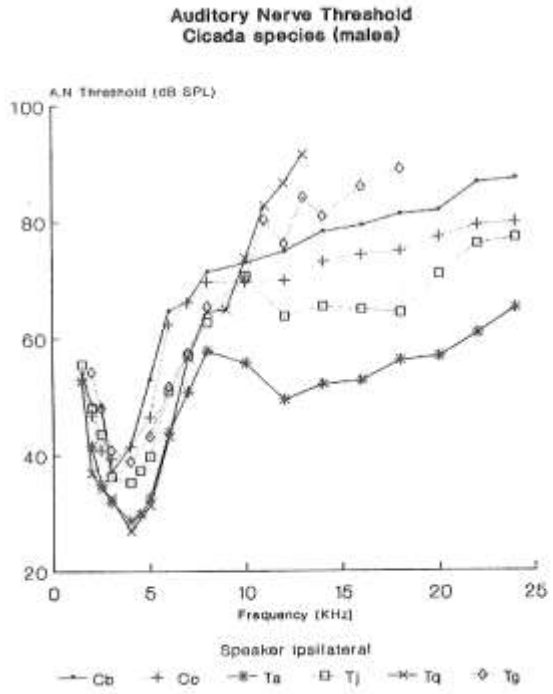


Figure 92 - Average hearing thresholds of male cicadas, estimated by monitoring with headphones the amplified summed auditory nerve responses to pure tone stimuli. All six species exhibited the lower thresholds around 3-5 kHz. Filogenetic related species showed similar patterns of the threshold curve. Cb *Cicada barbara lusitanica*; Co *Cicada orni*; Ta *Tettigetta argentata/atra*; Tj *Tettigetta josei*; Tq *Tibicina quadrisignata*; Tg *Tympanistalna gastrica*. Calculations were made on linear scale.

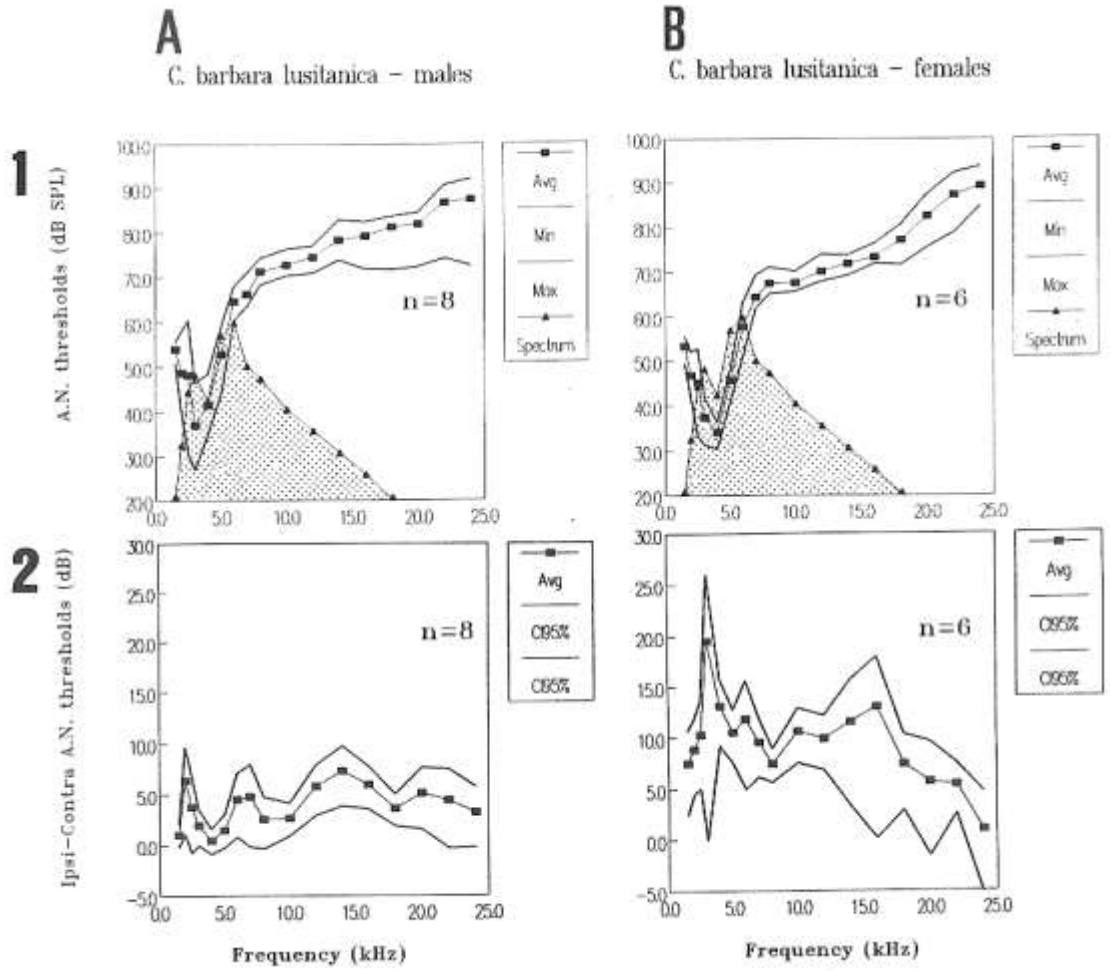


Figure 93 - Hearing on males (A) and females (B) of *Cicada barbara lusitanica*, estimated by monitoring with headphones the amplified summed auditory nerve responses to pure tone stimuli. 1) Average hearing thresholds and ranges of variation measured with ipsilateral sound, superimposed to a sampled spectrum of the conspecific calling song. 2) Frequency dependent directional hearing expressed as an average from the threshold differences measured with sound ipsi- and contralateral. The variation is explicited as 95% confidence intervals. (8 male and 6 female recordings). Calculations were made on linear scale. Notice that the logarithmic dB scale makes the upward confidence intervals look smaller than the downward ones.

species, presented the higher A.N. thresholds (Fig. 98 A1, B1). Sometimes it was possible to find large reversible differences in sensitivity (more than 10-15 dB) in the same animal and at the same frequency, indicating that cicadas can change the state of their hearing structures, and thus their sensitivity, probably due to the detensor tympani system (Pringle 1954).

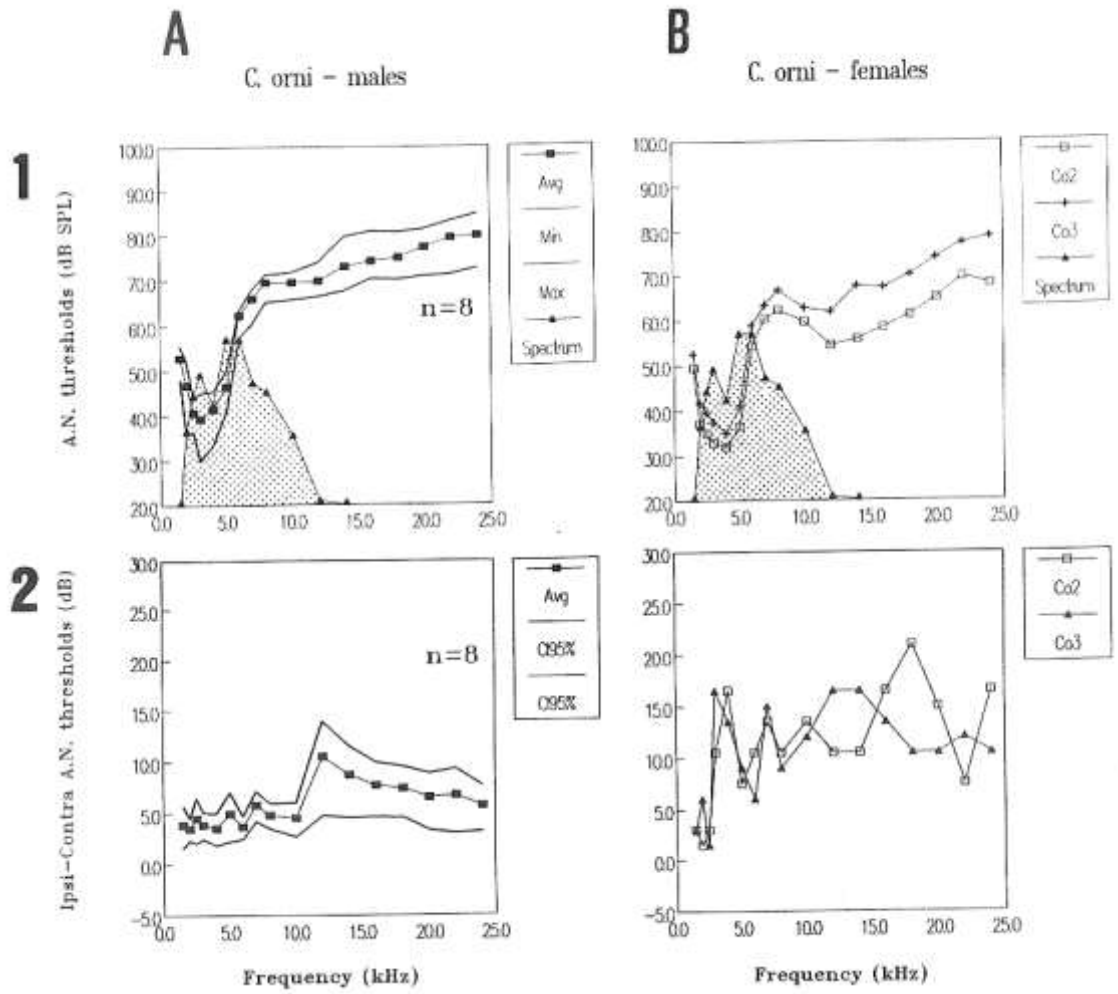


Figure 94 - Hearing on males (A) and females (B) of *Cicada orni*, estimated by monitoring with headphones the amplified summed auditory nerve responses to pure tone stimuli. 1) Average hearing thresholds and ranges of variation measured with ipsilateral sound, superimposed to a sampled spectrum of the conspecific calling song. 2) Frequency dependent directional hearing expressed by the threshold differences measured with sound ipsi- and contralateral (average differences for the males). The variation is explicited, for males, as 95% confidence intervals. (8 male and 2 female recordings). Calculations were made on linear scale. Notice that the logarithmic dB scale makes the upward confidence intervals look smaller than the downward ones.

At frequencies above 10 kHz the hearing sensitivity was very different among the species (cf. Fig. 92). While in the males of *Tib. quadrisignata*, with the steeper roll off towards higher frequencies, the threshold reached values above 90 dB SPL at 13 kHz, in the other extreme *Tett. argentata/atra* exhibited thresholds around 50 dB SPL at 12-14 kHz and responded up to 35 kHz or even more.

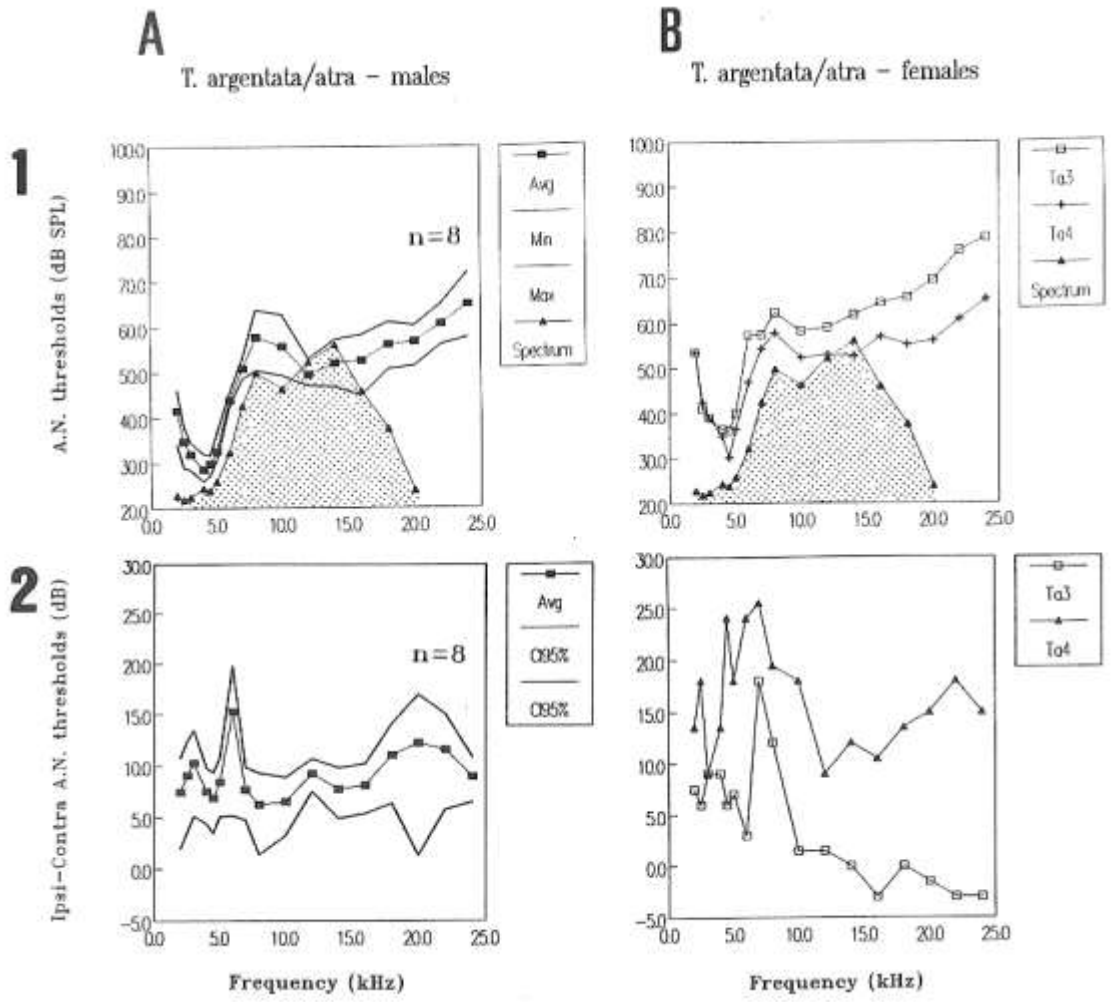


Figure 95 - Hearing on males (A) and females (B) of *Tettigetta argentata/atra*, estimated by monitoring with headphones the amplified summed auditory nerve responses to pure tone stimuli. 1) Average hearing thresholds and ranges of variation measured with ipsilateral sound, superimposed to a sampled spectrum of the conspecific calling song. 2) Frequency dependent directional hearing expressed by the threshold differences measured with sound ipsi- and contralateral (average differences for the males). The variation is explicited, for males, as 95% confidence intervals. (8 male and 2 female recordings). Calculations were made on linear scale. Notice that the logarithmic dB scale makes the upward confidence intervals look smaller than the downward ones.

The hearing sensitivity might change with the sex. This was the case of *Tymp. gastrica*, where males were 10 dB or even more sensitive than females at the frequencies with the lowest thresholds (Fig. 98 A1, B1; see also vibrational differences in Fig. 72). However, at higher frequencies the sensitivities became similar. In all other species males and females were more or less similar in their hearing optima. Sex differences at higher frequencies were also found. Females of *Tib. quadrisignata* responded up to 18-20 kHz (Fig. 97 B2) while males only reached 13-14 kHz (Fig. 97 B1) (at 13 kHz the females were 10-15 dB more sensitive than the males). Also in *C. orni* the females seemed to be more sensitive than the males at frequencies above 8 kHz (Fig. 94 A1, B1).

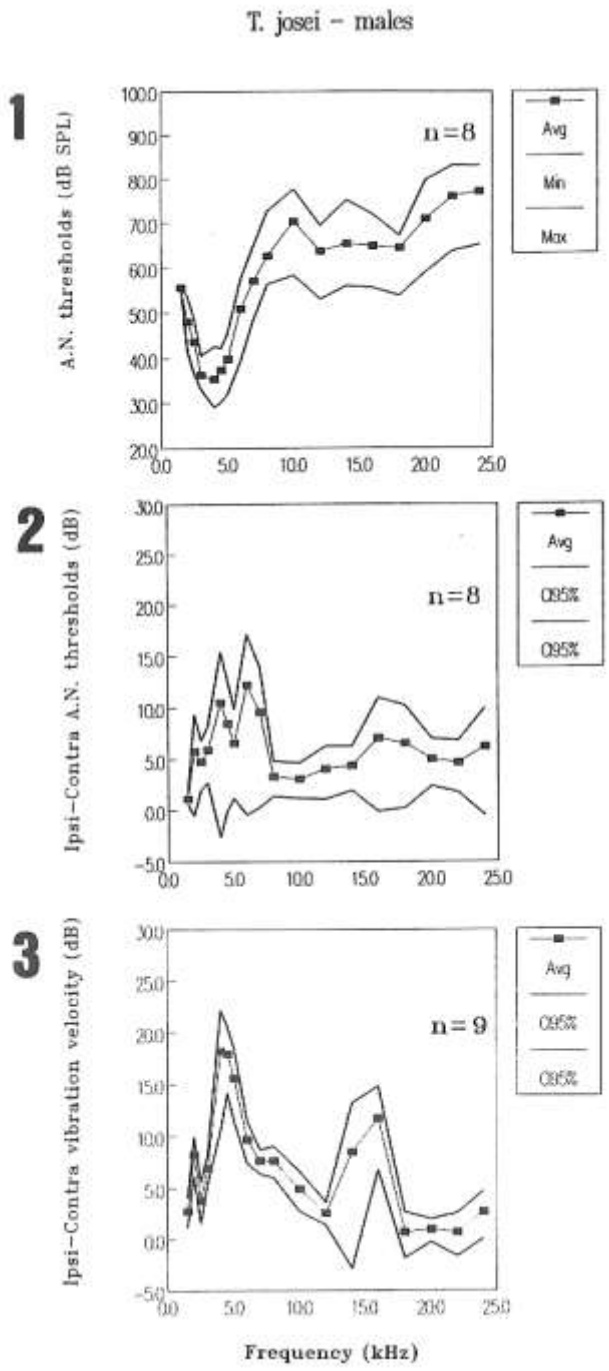


Figure 96 - Hearing on males of Tettigetta josei. 1) Average hearing thresholds and range of variation measured with ipsilateral sound. The thresholds were estimated by monitoring with headphones the amplified summed auditory nerve responses to pure tone stimuli (8 male recordings). 2) Frequency dependent directional hearing expressed as an average from the threshold differences measured with sound ipsi- and contralateral. 3) Frequency dependent directionality of the vibrations measured on the tympanic ridge, expressed as an average of the vibration velocity differences measured with sound ipsi- and contralateral (9 male recordings). The variation in 2) and 3) is explicited as 95% confidence intervals. Calculations were made on linear scale. Notice that the logarithmic dB scale makes the upward confidence intervals look smaller than the downward ones.

2. Hearing sensitivity and song spectra. As stated above, the frequencies of best hearing sensitivity seemed to be independent from the conspecific calling song. While in *C. barbara lusitanica* and *C. orni* (Figs. 93-1, 94-1) a good correspondence between hearing optimum and song spectrum was found, in *Tett. argentata* (*Tett. josei*) and *Tib. quadrisignata* there was a mismatch with the calling song spectrum (Figs. 95-1, 96-1, 97-1). In *Tymp. gastrica* there was a mismatch with the spectrum of the soft sound pulses (cf. Figs. 61 and 98-1) but the

correspondence improved when comparing the A.N. threshold curves with the spectrum of the loud sound pulses occurring at the beginning of each echeme of the calling and courtship songs (cf. Fig. 61).

It shall be noted, however, that the spectrum of the alarm signal of *Tib. quadrisignata*, with frequency components below the ones observed in the calling song, has a much better correspondence to the hearing sensitivity of this cicada. An alarm signal with the same intensity as the calling song could be perceived from a distance about 8 times greater (considering an attenuation factor of about 6 dB/double distance -- cf. spectra on Fig. 20 with the threshold curves on Fig. 97-1). Moreover, in *C. barbara lusitanica* and *C. orni* there was an increase at lower frequencies during the alarm signals, leading to a better matching with the A.N. threshold curve than it is observed in the calling song (cf. Figs. 93-1 and 94-1 with the song spectra on Figs. 10 and 12).

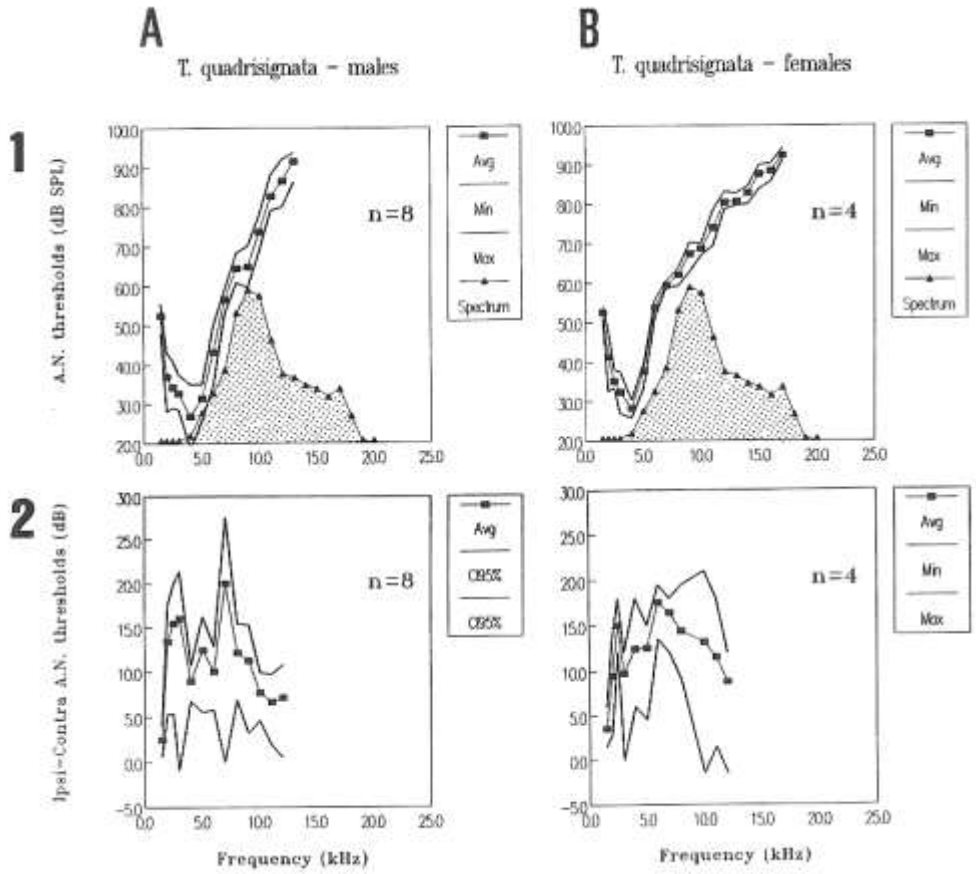


Figure 97 - Hearing on males (A) and females (B) of *Tibicina quadrisignata*, estimated by monitoring with headphones the amplified summed auditory nerve responses to pure tone stimuli. 1) Average hearing thresholds and ranges of variation measured with ipsilateral sound, superimposed to a sampled spectrum of the conspecific calling song. 2) Frequency dependent directional hearing expressed as an average from the threshold differences measured with sound ipsi- and contralateral. The variation is explicited as 95% confidence intervals. (8 male and 4 female recordings). Calculations were made on linear scale. Notice that the logarithmic dB scale makes the upward confidence intervals look smaller than the downward ones.

3. Directional hearing. The directional hearing capabilities were estimated by comparing the auditory nerve threshold curves obtained with sound presented ipsilateral and contralateral to the ear under study (Figs. 93-98, 2). Vibrational directionality of the tympanic membranes was also obtained for *Tymp. gastrica* and *Tett. josei* (Figs. 98-3, 96-3).

Low frequency directionality around the region of higher hearing sensitivity (2.5-6 kHz) was found in males and females of all species but for the males of *C. orni* (Fig. 94 A2) and to a less extent *C. barbara lusitanica* (Fig. 93 A2). Ipsi- to contralateral differences in this frequency region have frequently values around 10 dB and may reach 20 dB. Regions of high directionality were also found at higher frequencies. In the males of *Tymp. gastrica*, *Tett. josei*, and possibly *Tib. quadrisignata*, there was a peak in the directionality around the maximum of the calling song spectrum (12 kHz in *Tymp. gastrica*, 16 kHz in *Tett. josei* and 7 kHz in *Tib. quadrisignata* where it is a frequency intense in the alarm signal but less intense in the calling song) (cf. Figs. 98 A2, 96-2, 97 A2). The high variability found at these frequency regions may be associated with frequencies at which the tympanum do not vibrate much, and thus creating very localized minima in the spectrum of the tympanic vibrations, as was found in the males of *Tymp. gastrica* (cf. Fig. 75 A) and also in the males of *Tett. josei*, with sound contralateral.

Males and females of *C. barbara lusitanica* and *C. orni* showed very different directionality patterns. While the ipsi- to contralateral differences were large in females, presenting two directionality peaks at about 3 and 6-7 kHz (Fig. 93 B2, 94 B2) in good agreement with the song spectra and with the maximum hearing sensitivity (3 kHz), males exhibited much lower directionality, especially in *C. orni*, usually below 5 dB at such frequencies (Fig. 93 A2, 94 A2). At higher frequencies the males of *C. orni* might show some directionality, especially around 12 kHz (Fig. 94 A2). Females of both species also presented regions of high directionality above 10 kHz (Figs. 93 B2, 94 B2). The males and females of the other species (*Tett. argentata*, *Tib. quadrisignata* and *Tymp. gastrica*) in addition to a good hearing directionality at frequencies where the ear was most sensitive, they also exhibited directionality at frequencies corresponding to intense components of the song spectrum (Figs. 95 B2, 97 B2, 98 B2).

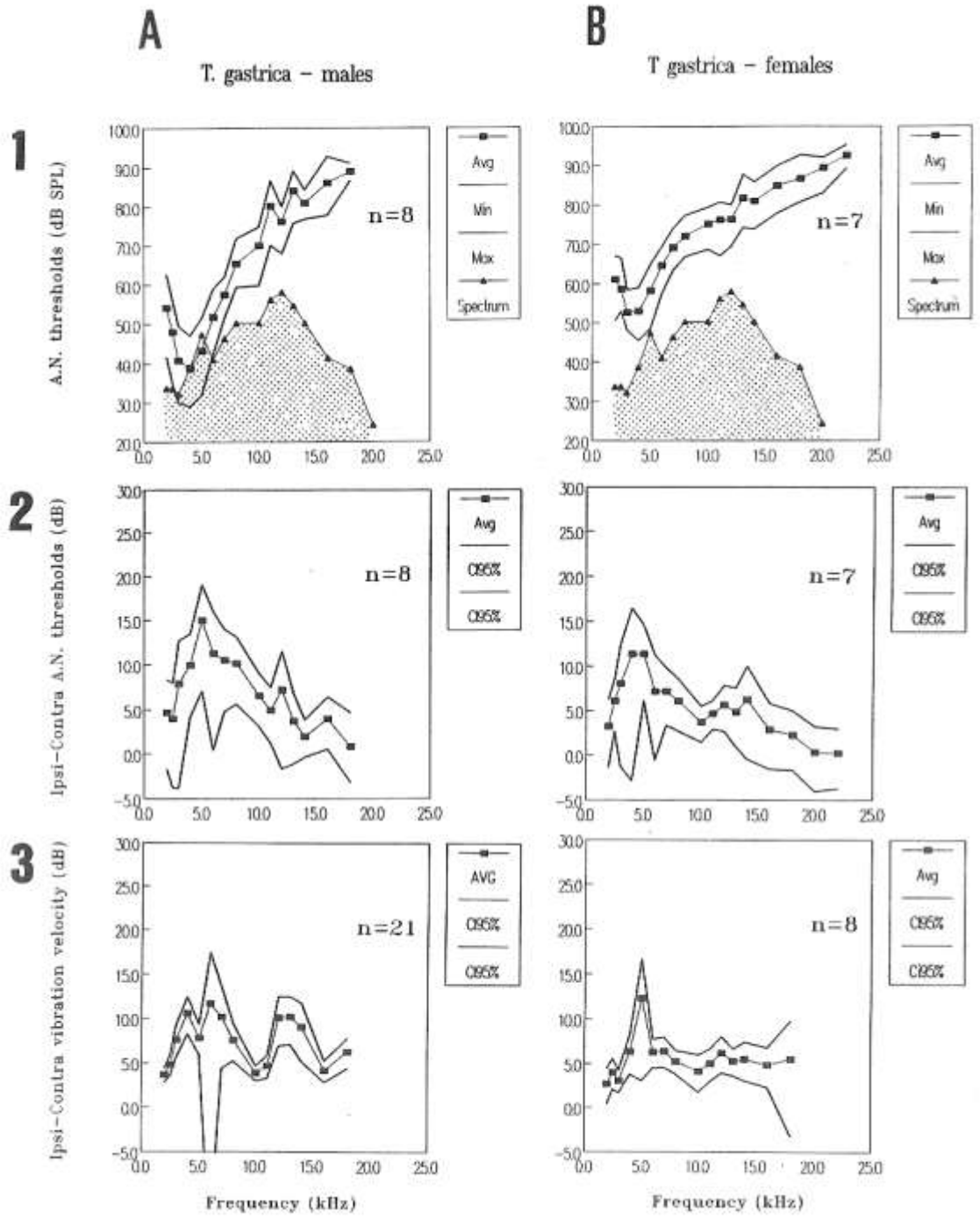


Figure 98 - Hearing on males (A) and females (B) of *Tympanistalna gastrica*. 1) Average hearing thresholds and ranges of variation measured with ipsilateral sound, superimposed to a sampled spectrum of the conspecific calling song. The thresholds were estimated by monitoring with headphones the amplified summed auditory nerve responses to pure tone stimuli (8 male and 7 female recordings). 2) Frequency dependent directional hearing expressed as an average from the threshold differences measured with sound ipsi- and contralateral. 3) Frequency dependent directionality of the vibrations measured on the tympanic ridge, expressed as an average of the vibration velocity differences measured with sound ipsi- and contralateral (21 male and 8 female recordings). The variation in 2) and 3) is explicited as 95% confidence intervals. Calculations were made on linear scale. Notice that the logarithmic dB scale makes the upward confidence intervals look smaller than the downward ones.

In *Tymp. gastrica* and *Tett. josei* it was possible to compare the vibrational directionality measured on the tympanic membrane with the directivity pattern exhibited after transducing by the auditory organ (Figs. 96-2,3, 98-2,3). The ipsi- to contralateral differences were comparable

in both cases. Moreover, the frequency ranges where the larger ipsi- to contralateral differences were measured presented a good correspondence between vibrational and auditory nerve data as well.

The mechanisms responsible for directional hearing were studied in detail in *Tymp. gastrica* (page 186). By having a glance on Fig. 99, which shows the sound scattering effects produced by the bodies of *Tymp. gastrica* (a small cicada) and *C. barbara lusitanica* (a medium sized cicada), one sees immediately that the directionality has to be created by some other process, especially below 10 kHz where scattering may be responsible only for less than 4 dB even in the large species. Probably the mechanism creating the large directionality patterns at low frequencies is similar in all these cicadas to the one showed in *Tymp. gastrica*.

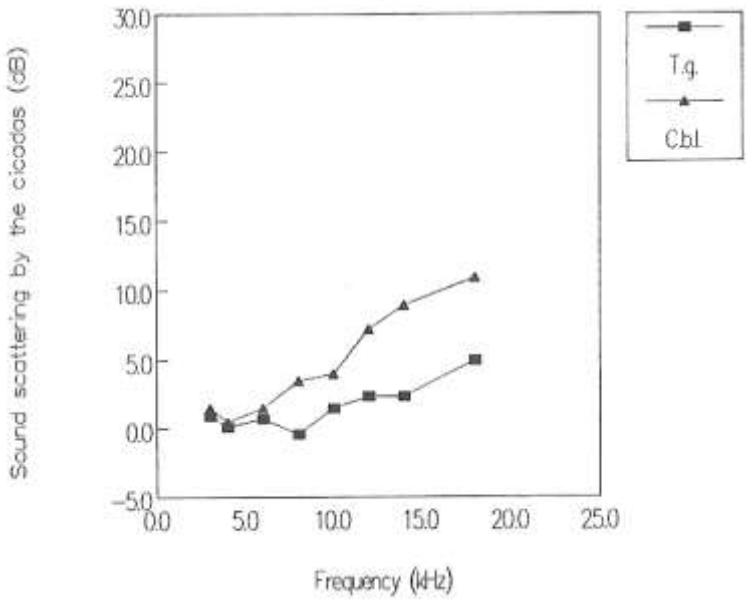


Figure 99 - Scattering of the sound waves produced by the body of *Tympanistalna gastrica* (Tg), a small cicada, and *Cicada barbara lusitanica* (Cbl), a medium size species. The plots represent the differences between ipsi and contralateral sound stimulation, with respect to the probe microphone position, on the sound pressure measured with the tip of the probe at about 1 mm from the lateral side of the cicada, close to the tympanum.

Discussion. The hearing sensitivity was estimated judging by ear the thresholds of the signals detected on the auditory nerve correlated with a pure tone sound stimulus presented to the animals. This method of estimating the thresholds has at least two disadvantages: 1) It is a subjective method greatly influenced by the background noise (nerve activity) in the whole nerve recording; 2) In species with a large number of receptors it is possible that the summed signal in the whole nerve recordings may be reduced for receptor cells whose frequency dependent sensitivity is considerably different from the majority of the cell receptor population. Therefore, the

results based on this method shall be considered with care (see also the discussion on threshold criteria in Michelsen 1971).

In general, the maximum sensitivity of the cicadas studied here, although showing large differences among the species, is in accordance with the range of sensitivities found in other cicadas (10 to 40 dB SPL) (Simmons et. al. 1971; Young and Hill 1977; Popov et. al. 1985, 1991; Popov and Sergeieva 1987; Popov 1989, 1990b; Huber at al. 1990). The frequencies at which the species proved more sensitive are also within the low frequency range where the majority of cicadas exhibit their lower hearing thresholds (e.g. Katsuki and Suga 1960; Simmons et. al. 1971; Popov 1990b). This feature alone suggests that cicadas shall have advantage in keeping a high sensitivity to low frequency sounds.

The hearing sensitivity might change in an experiment. Sometimes large variations in threshold occurred in a very short time, indicating that the animal was able to modify its sensitivity. To modulate the hearing sensitivity the cicadas possibly use their detensor tympani muscles, as was shown by Hennig et. al. (1994b) in some north American cicadas, or induce other modifications on the system, such as changes on the abdomen posture (Fonseca, 1993).

Related species belonging to the same genus did show a similar pattern on the auditory nerve threshold curve (cf. Fig. 92). This similarity may be structurally dependent (tympana with the same construction, similar sound transducer), and may reflect a common evolutionary history. If it is the mechanics of the ear or the transduction by the nervous system which are responsible for the low frequency similarities we do not know. This feature might be related to similar natural selection pressures (same predators, same communication constraints) since the curves are otherwise quite diverse in threshold pattern, or to phylogenetic reasons. It is likely, however, that both adaptive and phylogenetic reasons were responsible for shaping the characteristics of the tunning curves. The threshold curves obtained here for the males of *C. orni* are similar to the values found by Popov et. al. (1991) at the best hearing frequencies. However, above 10 kHz the Portuguese specimens were, on average, about 10 dB less sensitive than the animals studied by those authors.

The problem concerning the mismatch of the sender's signal by the capabilities of the acoustic receiver is discussed on page 259.

The frequency range corresponding to a higher hearing sensitivity was better matched by the spectra of the alarm signals than by the calling song ones in *C. barbara lusitanica*, *C. orni* and *Tib. quadrisignata* (cf. Figs. 92-1, 93-1 and 97-1 with Figs. 10 A,B, 12 and 20), species that readily produce a loud noise when disturbed. Moreover, the hearing directionality was, in most cases, good at frequencies corresponding to high energy components in the alarm signals, probably due in males to the sound input through the timbals (as shown previously in *Tymp. gastrica*). These observations on the sensitivity and directionality of the auditory receptor were unexpected and suggest a possible role of the alarm signal in the communication of danger situations among cicadas. The fact that in some species the ear is more sensitive at frequencies well represented in the alarm signal and below the calling song peak, may allow the detection, and even location of the source of the alarm signals and other lower frequency sounds in an acoustic environment dominated by the conspecific calling song.

The hearing directionality shown by the males of *C. barbara lusitanica* and *C. orni* is very different from the other cicadas. They exhibit a much smaller directionality at the frequencies corresponding to the maximum hearing sensitivity, an observation that shall be related to a different acoustics. The males of these two species are very similar, and both produce sound with relatively low frequencies. It is possible that some resonant properties of the abdomen may reduce the directionality characteristics of the hearing apparatus in these species. This was the case in the Australian cicada Cystosoma saundersii (Simmons and Young 1978; Fletcher and Hill 1978) where males lack directionality at the unusual low frequency of the calling song (around 800 Hz) due to the resonant properties of the male abdomen.

In *Tymp. gastrica* is possible to compare the vibrations of the tympanum with the threshold curves measured in the auditory nerve, and thus to see how is the information picked up by the tympana encoded by the auditory organ. The auditory threshold curves (Fig. 98) and the spectra of the tympanic vibration velocity (Fig. 72) are not very similar. The threshold curve is tuned to about 3-5 kHz whereas the vibrations have their maximum around 5-8 kHz. Furthermore, above 4 kHz the threshold increases rapidly with the frequency (from 5 to 10 kHz the threshold increases approximately 25-30 dB in males and 15-20 dB in females). In contrast, the vibration velocity is almost independent of the frequency between 5 and 10 kHz. The directionality observed in the

vibrations of the male and female tympana is encoded in the auditory nerve thresholds (cf. Fig. 98-2,3). Although some variations were detected, especially in the male at 5 kHz where a reduction in the mechanical directionality was not represented in the mean auditory threshold. Such variations might be related to the emplacement of the narrow dips detected with contralateral sound stimulation, which may vary in frequency among the animals, and might be undetected in a discrete measurement such as the one used to evaluate the physiological thresholds.

The discrepancies of the tympanal tuning found between laser measurements and nerve recordings could be due to: a) a resonant lever system connecting the tympanum to the auditory organ (Fletcher and Hill 1978), which might change the transduction tuning from the tympanum to the receptors, or b) provided that there might be different classes of receptors tuned to different frequencies, the receptor cells sensitive to lower frequencies could occur in larger numbers than those receptor cells sensitive to higher frequencies (i.e. > 5 kHz) which could bias the summed response recorded in the auditory nerve.

6. Central processing of auditory information.

The analysis of the auditory system and the auditory pathway of cicadas in this study up to this point has concentrated on the peripheral structures, the peripheral frequency filtering mechanisms, and whole nerve recordings describing the summed response of the auditory receptors. The methods employed allow important answers to questions posed at the onset of this investigation such as sensitivity and frequency tuning of the cicada ear, the mechanisms of directional hearing, and sex specific differences in the auditory system of cicadas. There are, however, also clear limitations of those methods since they do not allow conclusive answers to questions such as the ability for frequency discrimination, the possible mismatch of the auditory capabilities and the song spectrum, and the ability and mechanisms by which cicadas may be able to identify a conspecific sound signal. Answers to those questions can only be obtained by taking intracellular recordings from single and identifiable neurons within the MAC. Considering the complexity of the central auditory neuropile and associated with that complexity the probably large number of neurons processing acoustic information in cicadas, it is well beyond the scope of this study to undertake a complete and comparative investigation. However, the technique of single cell recording and staining may provide evidence helping to answer some of the questions outlined at the beginning, even though the results presented here are restricted to one species (*C. barbara lusitanica*). Hence, the following two sections will describe firstly the structure and morphology of the auditory interneurons in relation to the auditory neuropile described previously (Chapter 4) and secondly, the physiology of those interneurons and their response characteristics to sound and, more specifically, to the pattern of the songs of *C. barbara lusitanica*.

6a. Morphology of identified MAC neurons.

Twelve different types of auditory interneurons recorded intracellularly in *C. barbara lusitanica* were classified upon their morphologies (Fig. 100). The cells were distinguished on the basis of the cell body position, the course of the primary neurite, the distribution of the dendritic

arborizations in the neuropile region (i.e. the receptor projections within the metathoracic abdominal ganglionic complex -- MAC), and the side of the ascending axon, ipsi- or contralateral, with respect to the cell body position. All the cells included in this chapter show ascending axons from the MAC. The neurons responded to sound. Most of their dendritic arborizations were in the region of the auditory neuropile whose outline was previously obtained in backfills and which was superimposed on the drawings shown on Figure 100. To name the neurons, the nomenclature on Robertson and Pearson (1983) was used. Moreover, the prefix AN was employed for auditory neuron. These results must be considered preliminary, since in most cases the cells were found, recorded, and stained only once, and therefore it is not possible to evaluate how representative their responses are, and how complete the cell staining was. Nevertheless, I think it is justified to include them on this work since the data available on cicada auditory neurons is very scarce. Previous work was presented by Huber et. al. (1980, 1990).

(AN_501 to AN_504 (Fig. 100), and AN_505 (Fig. 101 E): these cells belong to a class where the soma is located far laterally in the anterior metathoracic region of the MAC. The primary neurite crosses the ganglionic midline and the ascending axon together with the dendritic fields develop only in the contralateral hemiganglion. The arborization fields are located posterior to the cell body and within the region of the auditory neuropile. The arborizations may be dense and most of them are situated in the metathoracic region. A few arborizations are also usually present in the mesothoracic ganglion. The morphological differences among these cells are seen in the main ramifications of the dendritic region. While in AN_501 there is a thick branch which divides posteriorly in two other main branches, in AN_504 there are two main branches descending already from the primary neurite. In AN_502 and AN_503 there is only one thick descending branch, and most of the ramifications are to the lateral side. In contrast, AN_505 sends branches also to the midline. In this case the cell body is also in a less lateral position than in the other cells of this morphological class.

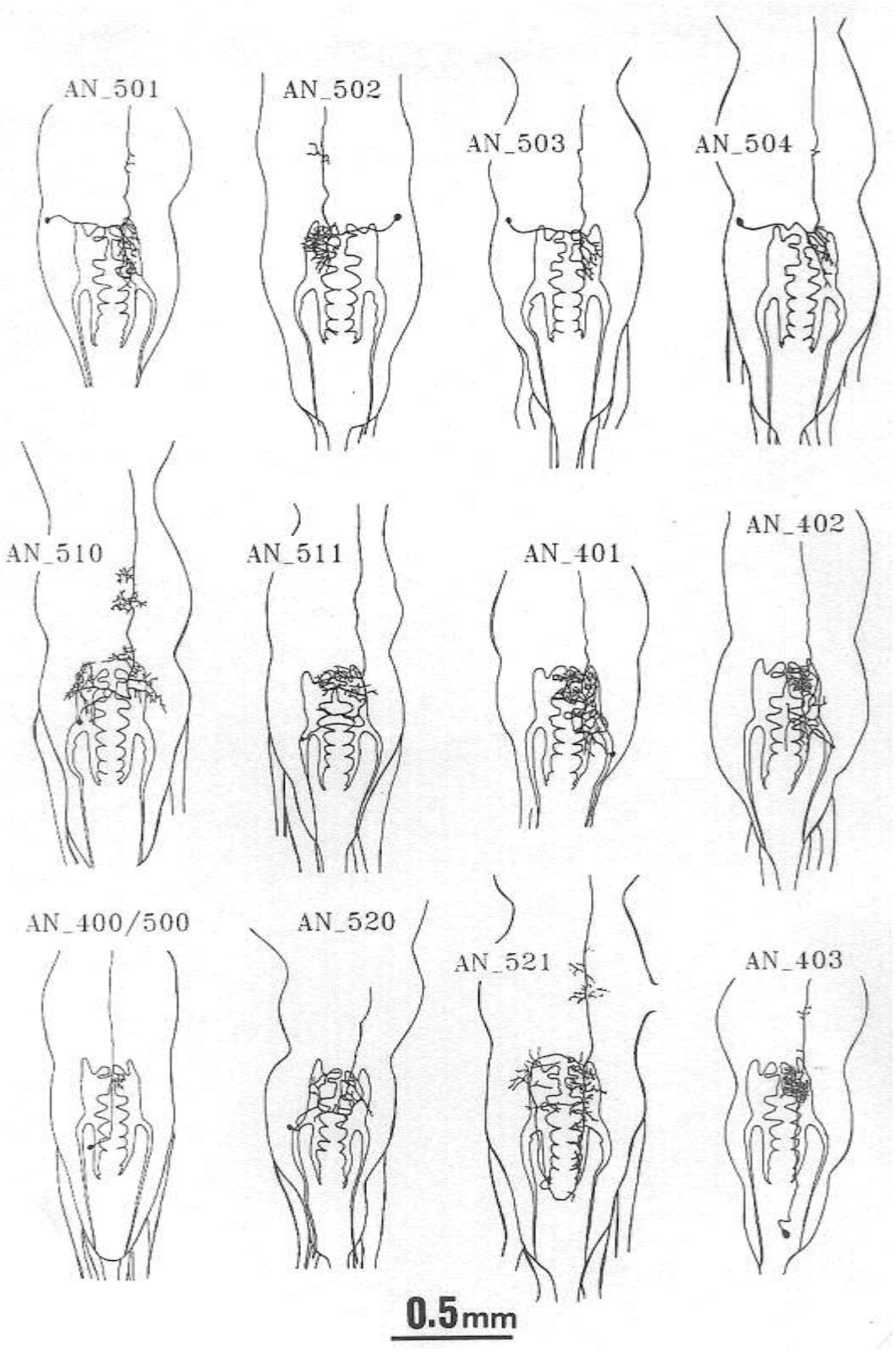


Figure 100 - Morphology of identified ascending auditory interneurons in *Cicada barbara lusitanica*, stained within the metathoracic-abdominal ganglionic complex (MAC) by injection with Lucifer Yellow. The outline of the auditory neuropile was superimposed on the drawings.

AN_510 (Fig. 100): the soma is located anteriorly in the abdominal region of the ganglionic complex at midway from the midline to the lateral side. The dendritic fields, present in both hemispheres, are not confined to the auditory neuropile region and extend more to the periphery in both sides, suggesting that this one may be a multimodal cell. The primary neurite joins a thick transverse branch at the ganglionic midline. In the side ipsilateral to the soma this transverse branch originates some dendritic arborizations. In the contralateral hemisphere several dendritic fields arise only after the axon makes a turn and orientates anteriorly. One of these fields seem to cross the midline of the ganglionic complex. From this ascending contralateral axon at least two dendritic fields also originate within the mesothoracic ganglion, none of which seemed to cross the midline.

AN_511 (Fig. 100): The soma of this ascending interneuron, smaller than the soma of the majority of the cells observed, is located anteriorly in the abdominal region. The primary neurite originating from the cell body orientates transversally and turns anteriorly at the middle of the contralateral hemisphere to form the ascending axon. At least three arborization fields originate from this axon mostly in the metathoracic region, sending nearly all branches towards the midline which they also cross. No dendritic fields were seen in the mesothoracic ganglion. A similar cell was found in Magicicada by Huber et. al. (1990).

AN_401 and AN_402 (Fig. 100): In these cells the soma is located laterally in the middle of the abdominal region. In both cases the primary neurite projects anteriorly with an oblique orientation towards the midline where it makes a loop from which the ascending axon originates. The ascending axon remains ipsilateral to the cell body. The dendritic fields spread both in the abdominal and in the metathoracic regions, developing in both sides of the ascending branch, but they are clearly more dense in this last region. Here the dendritic fields clearly cross the midline in AN_401, but in AN_402 they stop approximately at about the midline. In both cells the ascending axon did not seem to form arborizations at the level of the mesothoracic ganglion.

AN_400/500 (Fig. 100): The soma is located in the abdominal region at the middle of the hemisphere. The primary neurite projects to the midline of the ganglia with an oblique orientation and then turns anteriorly. This auditory interneuron is unique in having the ascending axon at the midline of the ganglionic complex. There is just one small arborization field situated in the mesothoracic region of the auditory neuropile, and it spreads only towards the side opposite to the cell body. A second, similar cell was stained and this cell showed also a second smaller dendritic arborization in the mesothoracic ganglion, also developing towards the contralateral hemisphere.

AN_520 and AN_521 (Fig. 100): These U-shaped cells have branches in both hemispheres at the level of the auditory neuropile.

In AN_520 the cell body is in the middle of the abdominal region and at midway to the lateral side of the ganglionic complex. The neurite arising from the cell body connects to the U-shaped branches. These branches divide in each hemisphere but the arborization fields did not seem to be dense. The ascending axon is peculiar in connecting to the symmetrical centre of the U over the ganglionic midline, and then ascends at the contralateral side. A similar cell was found in Magicicada by Huber et. al. (1990).

In AN_521 the cell body is situated posterior in the abdominal region and more towards the midline. The U-shaped neuropil, where the short primary neurite is connected, and the dendritic arborizations cover most of the auditory neuropile with branches in both hemispheres. The dendritic fields are more abundant in the contralateral side. Ramifications are sent both towards the middle and the lateral side of the ganglionic complex, and some of the ramifications cross the midline. The end of the neuropil branch projecting at the soma ipsilateral side turns at the metathoracic region crossing the midline. The contralateral end of the U continues forming the contralateral ascending axon. Some dendritic fields could be recognized at both sides of the ascending axon in the mesothoracic ganglion.

AN_403 (Fig. 100): The soma of this cell is situated at the posterior tip of the MAC complex, nearly at the midline. The axon arising from the cell body makes a short loop before ascending along

the ipsilateral side nearly in a straight line. Most arborizations lie in the metathoracic auditory neuropile, where the branches do not seem to cross the ganglionic midline. A small region with arborizations was also detected in the mesothoracic ganglion.

6b. Response characteristics of MAC neurons

The single cell recordings were carried out in *C. barbara lusitanica* with a sound stimulus constituted either by a calling song sequence or by 60 ms pure tone pulses shaped both in the beginning and at the end by a 6-8 ms ramp. These stimuli were presented in trains of 5 pulses with 200 ms intervals, separated to the next train by a 500 ms pause, and could cover a frequency range from 2 to 35 kHz, at 40 to 110 dB SPL (re. 20 microPascal).

Among the questions addressed with such an intracellulary approach are: a) What is the correspondence between the auditory receptor thresholds as evidenced by the summed whole nerve recordings and the threshold curves of single auditory interneurons. b) What are the dynamic ranges of the cells. c) Are there indications for frequency discrimination; in another words, how broad is the tuning of the cells within the CNS and do different neurons cover different frequency ranges. d) How well does the tuning of the interneurons match the song spectrum. e) How faithfully is the calling song structure copied by single interneurons; how do the cells respond to typical time and amplitude patterns found in the songs of *C. barbara*, e.g. the slow increase in intensity and abrupt pauses.

Response characteristics of identified auditory interneurons. All stained auditory interneurons exhibited an ascending axon. Their physiological characteristics are summarized on Figure 101 and Table 8.

AN_501 to AN_507 (Fig. 101 A-G): All these T-shaped cells displayed a phasic-tonic response. Usually the response became tonic at moderate and high intensities above threshold, and sometimes it seemed also frequency dependent. The response was accompanied by a pronounced EPSP in all cases except for AN_503 (Fig. 101 C), where

Table 8 - Physiological characterization of the auditory ascending interneurons identified in Cicada barbara lusitanica.

Cell	Response type	PSP's EPSP	PSP's IPSP	Threshold curves Best frequency	Threshold curves Best sensitivity	Dynamic range	Adaptation at high intensity	Latency	Highest spiking rate	Type of intensity functions	Spontaneous activity -resp.	Some location	Most important dendritic fields and density
AN_L2 [511]	P-T	X	X	< 2 kHz	< 70 dB SPL	>= 25 dB	(s-m)	22 ms	235 s^{-1}	S	Y	A(a)	M (m)
AN_L4 [402]	T	No?	No	< 2	< 50/55	>= 25	(s-m)	26-28	145 (90)	C, S	Y	A(m)	M (m)
AN_L2 [521]	T	No	No	< 2	< 65	>= 10-15	(s)	22	140	S, B	Y	A(p)	M,A
AN_I1 [403]	T	X	?	< 2	< 50/55	>= 30-35	(m)	28	270	S	Y	A(p-tip)	M (m-h)
AN_T1 [501]	P-T	X	No	4	55	40-50	(m-h)	24-26	300	S	Y	M	M,Aa (m)
AN_T2 [502]	P-T	X	No	4, 18	55/60	30	(m)	22-24	420	S	Y	M	M,Aa (m-h)
AN_T3 [503]	P-T	X small	No	< 2?, 3	<= 65	>= 30	(s)	18-20	160	S, B	Y	M	M,Aa (m)
AN_T4 [504]	P-T	X	No	4	55/60	25-30	(s)	18-20	240	S	Y	M	M,Aa?
AN_T5 [505]	P-T	X	?	< 2?, 4	<= 60/65	>= 35	(m)	22	210	S, B?	Y	M	M (m?)
AN_T6 [506]	P-T	X	No	3-5,18-20,24	45/50	>= 20	(s-m)	22-24	250	S, B	Y	M	M,Aa? (m?)
AN_T7 [507]	P-T	X	?	< 2?, 2-3	<= 50/55	>= 30	(m)	20-22	220	S, B?	Y	M	M,A (m?)
AN_U1 [520]	P-T	X	No?	< 2?, 10	<= 65/70	>= 20-25	(s)	20-22	170	S	Y	A(a-m)	M (s)
AN_L1 [510]	P-T	X	No	10-15,24-35	70/75	15	(s)	22-24	250-300	S, B	No	A(a)	M (s)

only a small depolarization was observed. In this cell the highest spiking rate was around 160 s-1, clearly below the rate found on the other cells of this morphological class which ranged from 210-420 s-1 (Table 8). All these interneurons had a minimum in their threshold curves around 3-4 kHz with best sensitivities ranging from 45 to 60-65 dB SPL and dynamic range of about 25-35 dB. The exceptions were AN_506 with a dynamic range of only about 20 dB (Fig. 101 F) and AN_501 presenting the highest range of all cells, around 40-50 dB (Fig. 101 A). In most interneurons another best frequency range was present. While in AN_503 (Fig. 101 C), AN_505 (Fig. 101 E) and AN_507 (Fig. 101 G) there is a possibility that they exhibited another region of high sensitivity at frequencies below 2 kHz, in contrast AN_502 (Fig. 101 B) and AN_506 (Fig. 101 F) displayed another low threshold region around 18-20 kHz, which was as sensitive as the low frequency one. The latencies, measured from the onset of the sound stimulus to the caused depolarization of the cell, ranged from 18-20 ms to 24-26 ms (Table 8). All cells did show spontaneous activity and there was variable adaptation at high intensities. Adaptation observed in a five pulses train was small in AN_503 and AN_504 but stronger in AN_501. The intensity response functions were in most cases sigmoid (Table 8), but in some cases there could be a secondary reduction in the spiking rate with increasing sound intensity, originating in more complex intensity-response functions (e.g. AN_503, Fig. 101 C, and AN_506, Fig. 101 F).

AN_510 (Fig. 101 H): This cell was unique in having a predominant phasic response and in presenting lower thresholds at 10-15 kHz and again above 24 kHz, despite of exhibiting a maximum sensitivity of only 70-75 dB at 12 kHz. The dynamic range was very small (around 15 dB). The spontaneous activity and adaptation at high intensities were very small. The maximum spiking rate could reach 300 s-1. The suprathreshold latency was around 22-24 ms. The type of intensity response could vary with frequency.

AN_511 (Fig. 101 I): this L-shaped cell was first excited by the sound pulse but after the first spike there was an inhibitory hyperpolarization followed by an EPSP accompanied by phasic-tonic spike discharges. The best frequency range was below 2 kHz and the threshold increased to reach

values above 100 dB at 6 kHz. The highest spiking rate was around 240 s-1 and the latency was about 22 ms.

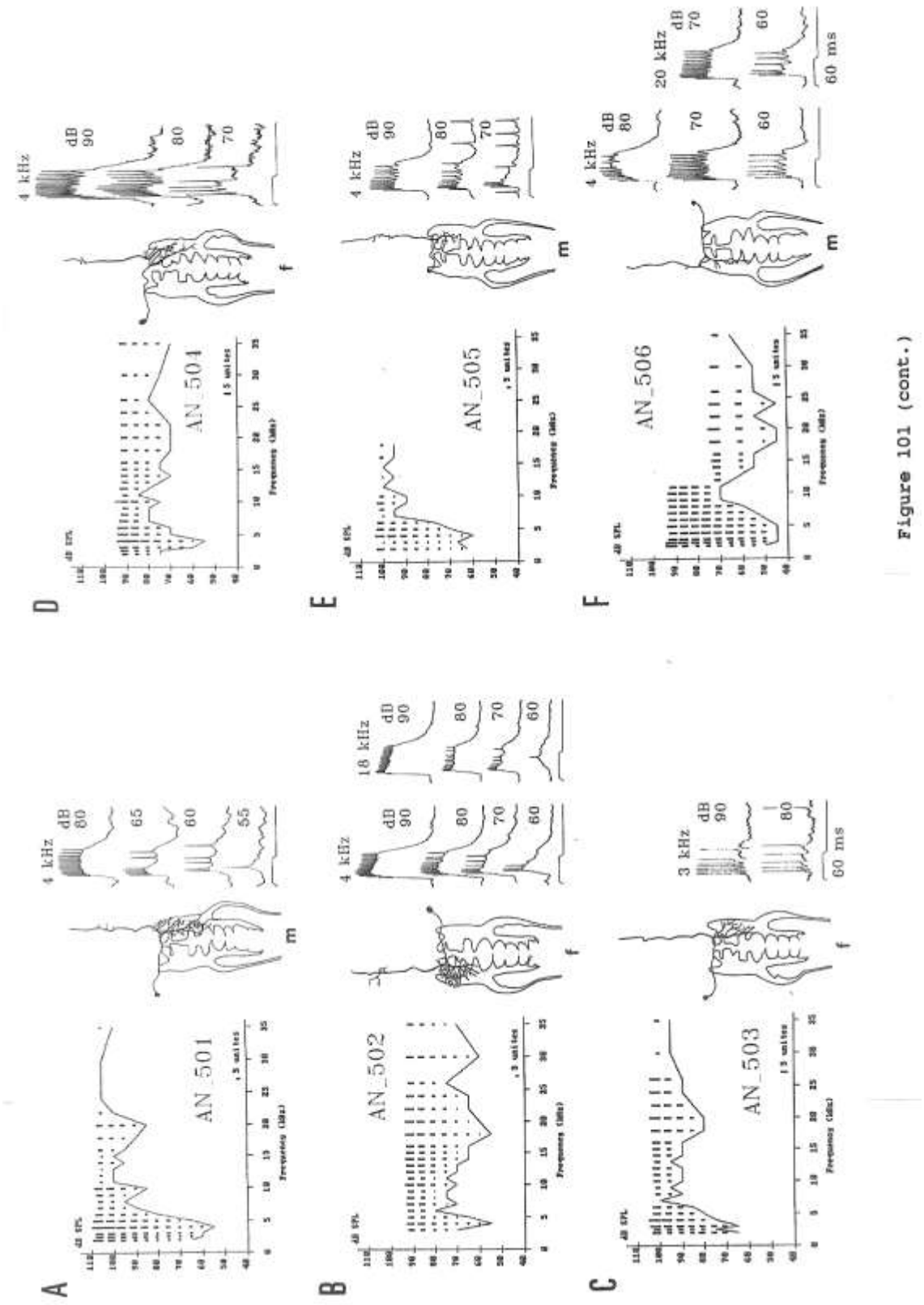


Figure 101 (cont.)

Figure 101_1 (cont.)

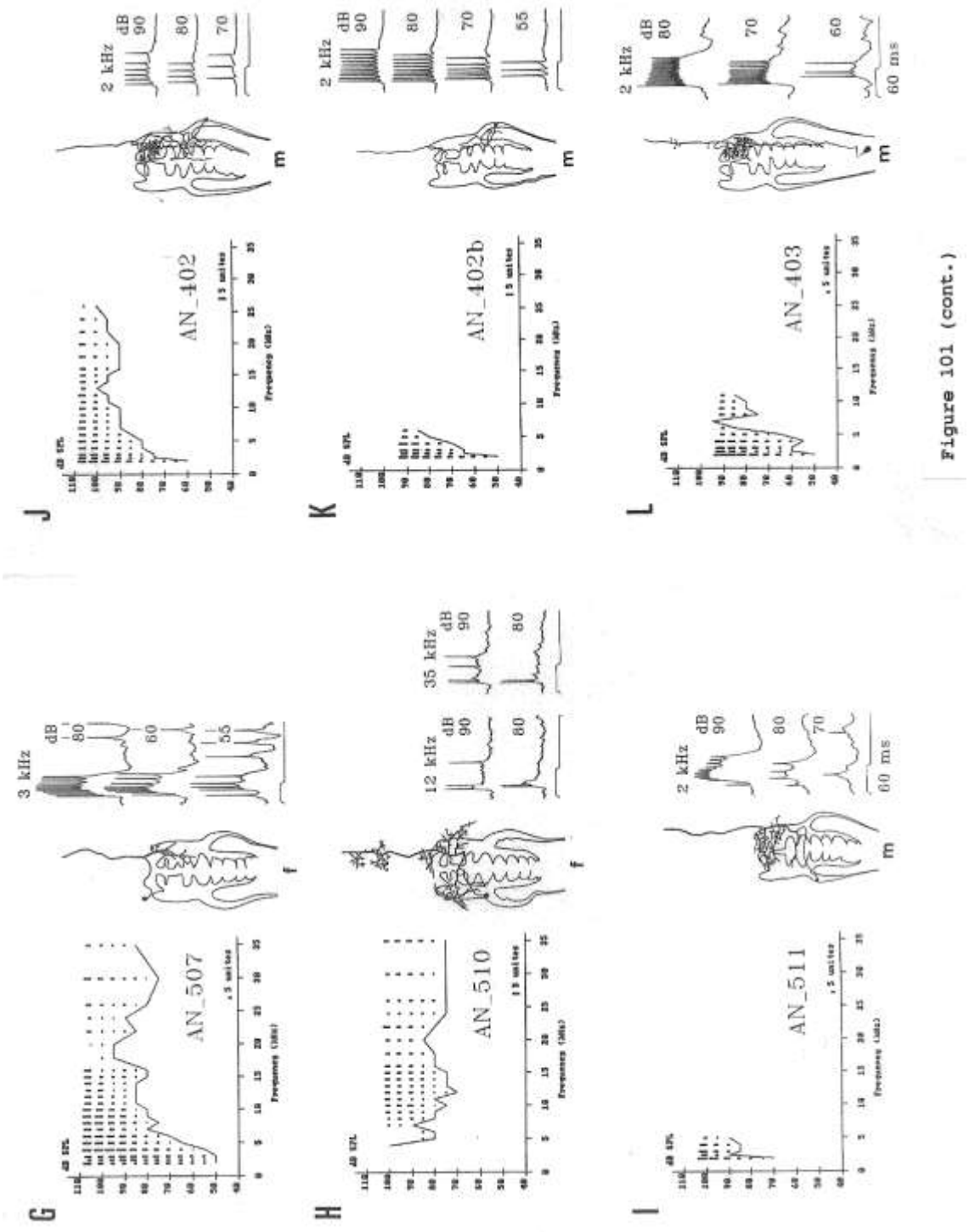


Figure 101 (cont.)

Figure 101_2 (cont.)

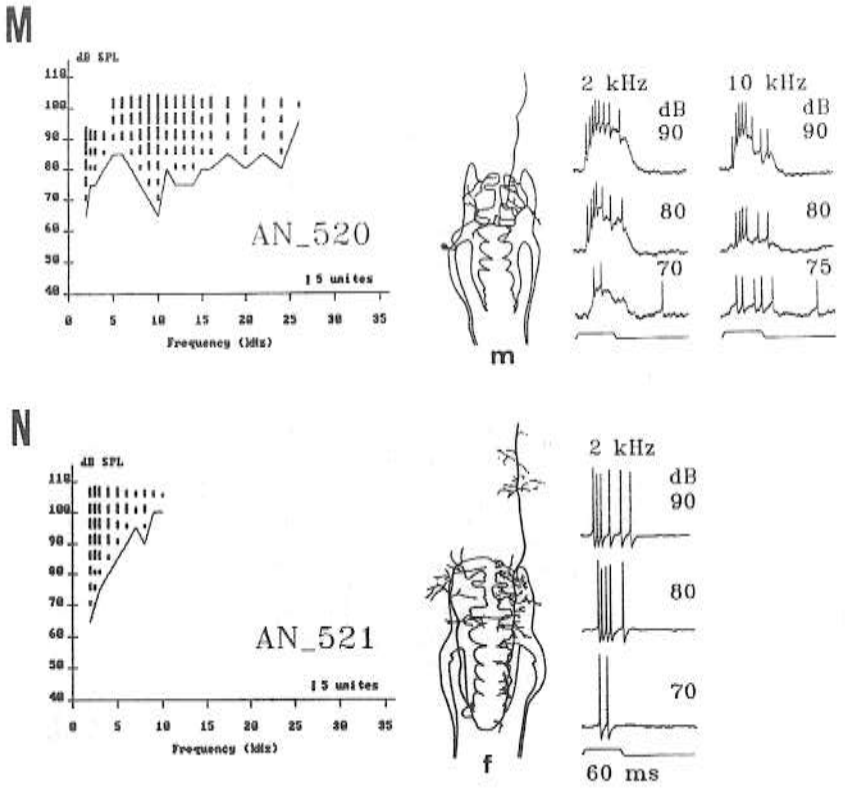


Figure 101 - Physiological characteristics of identified ascending auditory interneurons in *Cicada barbara lusitanica*. The plots represent the average number of spikes (presentation of 5 stimuli) correspondent to pure tone sound pulses with different amplitudes and frequencies. The threshold corresponds usually to an increase of one spike on the cell activity and was also judged by following visually the cell responses on chart. An example to the cells' reactions to pure tone 60 ms sound pulses at their best frequencies is shown as well. m male; f female. Further explanations on the text.

AN_402 (Fig. 101 J), AN_403 (Fig. 101 L), AN_521 (Fig. 101 N): These three cells showed a tonic response to the sound. All had their lowest thresholds below 2 kHz and a steep roll off towards higher frequencies. EPSPs were only present in the I-shaped AN_403, which had a higher spiking rate (about 270 s-1 for AN_403, and below 150 s-1 for the other two). The dynamic range was small in AN_521 (only about 15 dB) and 25-35 dB in the others. The latency was about 26-28 ms in AN_402 and AN_403 but approximately 22 ms in AN_521. The adaptation at high intensities was small in the U-shaped cell (AN_521) and variable in the other cells. The intensity response functions were sigmoid, and the spike rate might even decrease in some frequencies at high intensities (AN_521). In AN_403 the first spike was always clearly the larger (Fig. 101 L). This was not as evident in the other cells studied.

AN_520 (Fig. 101 M): This cell had a phasic tonic response. The threshold curve had a minimum around 10 kHz where the sensitivity was about 65 dB SPL. Another more sensitive region might

occur below 2 kHz. The dynamic range was about 20-25 dB and the latency was 20-22 ms. The highest spiking rate was 170 s-1. The intensity response functions were sigmoid.

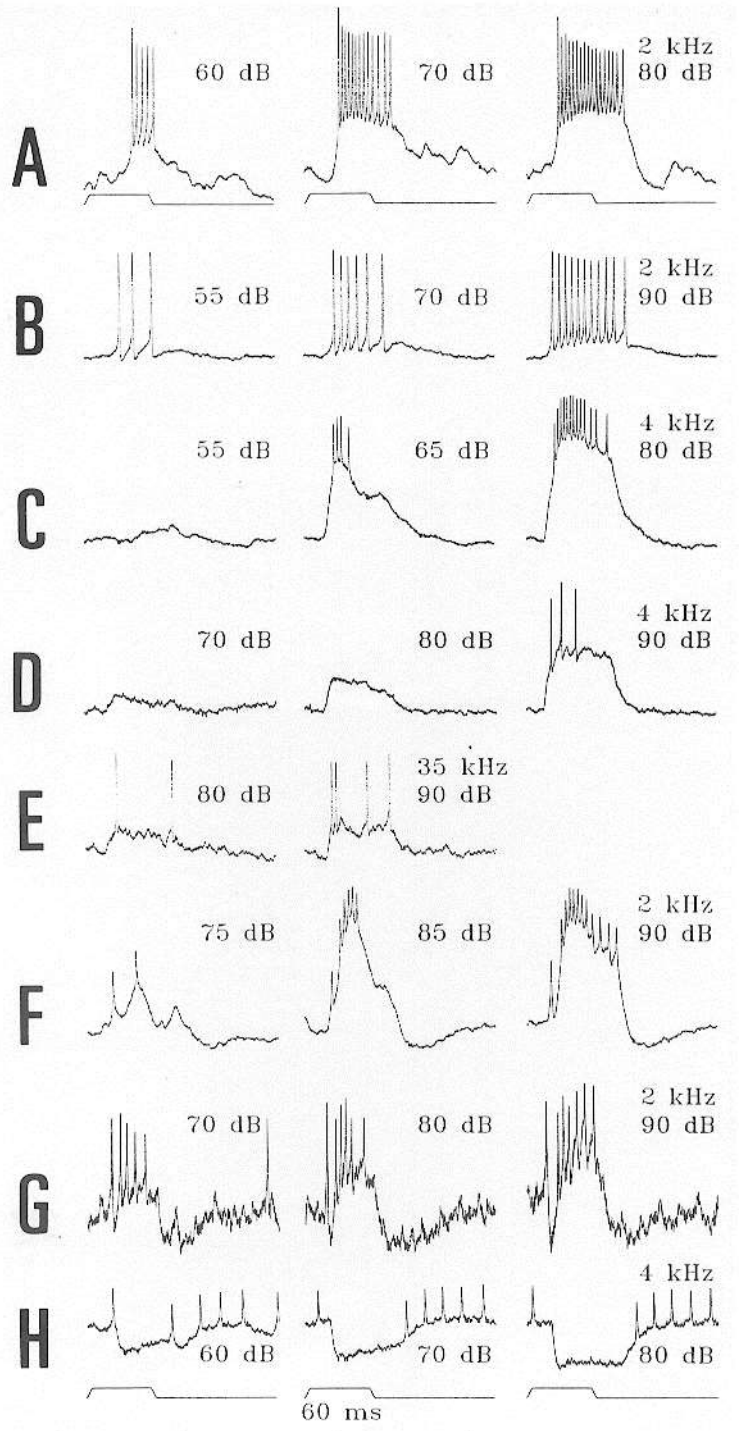


Figure 102 - Types of responses to pure tone sound stimuli found on auditory interneurons of *Cicada barbara lusitanica*. Excitatory responses: A,B) Tonic; C) Phasic-tonic; D,E) Phasic. F,G) Inhibitory IPSP followed by excitatory responses phasic-tonic (F) or tonic (G). H) Inhibitory response, during which the spontaneous cell activity was suppressed.

Three other neurons were recorded and stained, including two multimodal neurons responding to sound and one local pre-motor neuron most likely belonging to the sound production circuitry. However, as they do not add information to answer the problems posed in the foreword (page 176) and in the introduction to this chapter (page 237), this data was omitted from this thesis.

Types of cell responses. A general overview of the types of responses found in the auditory interneurons is exemplified on Figure 102. The majority of cells were excited by the pure tone sound pulses and most of the responses could be classified as phasic-tonic (Fig. 102 C). Some of the identified interneurons exhibited tonic responses. These reactions were in some cases accompanied by excitatory post synaptic potentials (EPSP) (Fig 102 A) while in other cases there were no prolonged cell depolarizations (Fig. 102 B). Phasic responses (Fig. 102 D) were very uncommon, however it was sometimes possible to observe a phasic response at the beginning and at the end of the stimulus (Fig. 102 E).

Inhibitory responses were also observed. Some unidentified cells presented a high continuous spontaneous activity which was inhibited during the sound presentation (Fig. 102 H). An inhibitory hyperpolarising post synaptic potential (IPSP) abolished the spontaneous spiking activity. Sometimes the cells presented a complex response with an IPSP following an initial excitatory reaction. After the short cell hyperpolarization either a tonic (Fig. 102 G) or a phasic-tonic (Fig. 102 F) spike discharge accompanied by an EPSP could be observed. In this way, such cells evidenced their multiple synaptic connections.

Comparative aspects.

a) Relationship between response type and frequency tuning. Most cells found had a phasic-tonic response dependent on intensity and/or frequency. The neurons showing small or no EPSPs had tonic responses and they also exhibited the lower spiking rates. All cells displaying a tonic response had their best frequency range below 2 kHz (Table 8). In contrast, the majority of interneurons with phasic-tonic responses were more sensitive at 3-5 kHz (Table 8). Cells which

were most sensitive at frequencies above 10 kHz were phasic-tonic or mainly phasic. In contrast, all tonic interneurons recorded have a fast roll off in sensitivity towards higher frequencies.

b) Relationship between arborization within the auditory neuropile and sensitivity. It appears that the cells presenting less dense dendritic fields in the metathoracic neuropile region may be less sensitive. The same was observed in Magicicada auditory interneurons (Huber et. al. 1990). There seems to be no clear correlation between the antero-posterior position of the cell body or the neuron morphology with the best frequency range. Based on this preliminary study there were no indications of a tonotopic organization within the auditory neuropile.

c) Intensity response characteristics. The intensity-response characteristics might vary in the same cell with frequency. Sigmoid shape with saturation was the most common situation (Fig. 101, and Table 8). Some cells might also show bell shaped intensity-response characteristics where, after a maximum number of spikes was achieved, the cell responded to higher intensity stimuli with a lower number of spikes.

Cells with different morphologies might show similar physiological characteristics (e.g. AN_402 and AN_521, or AN_505 and AN_520, Fig. 101). In contrast, cells with a similar morphology might have different physiologies (e.g. AN_502 and AN_507, Fig. 101).

Relation between Auditory nerve thresholds and interneuron thresholds. Many of the cells identified exhibited threshold curves which did not correspond to the hearing threshold obtained from whole auditory nerve recordings (Fig. 103 A). Regions of higher sensitivity than that displayed by the auditory nerve threshold curve were present in many cells either at low frequencies (below 3 kHz) or at higher frequencies (above 8-10 kHz). With more than one thousand receptor cells (1400) with axons in the auditory nerve, it is possible that a few receptors sensitive to other frequencies may escape detection in the recording of the whole auditory nerve. This situation suggests that the measurements in the whole auditory nerve may represent the most common receptor situation, but they cannot be representative for the complete sensitivity range of the auditory system.

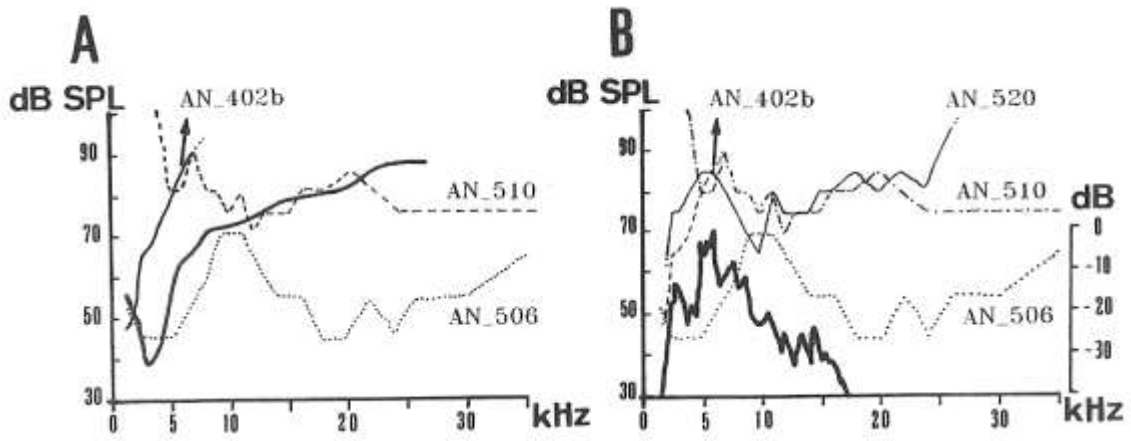


Figure 103 - Comparison between the thresholds of some auditory interneurons of *Cicada barbara lusitanica* and A) the hearing threshold (averaged male curve) estimated by the earphone method using the whole auditory nerve recordings, or B) the calling song spectrum. Neurons very sensitive at frequencies above 10 kHz, and also possibly below 2 kHz, are not evidenced in the estimated whole auditory nerve threshold. While some neurons match the calling song spectrum, as the curve for the whole auditory nerve does, other, or even the same neurons are sensitive at frequencies where the calling song has a low intensity, which is not shown by the threshold curve estimated with the summed responses on the auditory nerve.

Frequency tuning of interneurons in relation to the calling song spectrum. A commonly discussed problem in cicadas refers to the adjustment between best hearing frequencies and the maximum power in the spectra of the conspecific sound signals (e.g. Popov 1990b; Huber et. al. 1990). In view of the present data for *C. barbara lusitanica*, this discussion should not be based on auditory nerve threshold curves alone, at least in cicadas, since in a species where an auditory nerve threshold curve mismatch with the song spectra is found, it is possible that some cells are still very sensitive to the peak frequency of the song. While several cells found in the present species clearly match the peaks of the spectra of the signals (calling, alarm), some other cells were very sensitive below and above the frequencies well represented in the songs (Fig. 103 B). Thus, the tuning of single cells in *C. barbara lusitanica* evidences a hearing sensitivity which covers a broad frequency range. Moreover, and as was also found in Magicicada (Huber et. al. 1990), since different neurons are tuned to different frequencies, frequency discrimination is in principle possible within the central nervous system, although behavioural data suggesting such an ability is scarce (Doolan and Young 1989).

Auditory interneuron responses to the calling song.

Figure 104 shows the responses displayed by some auditory interneurons both to a play-back of a calling song sequence and to a pure tone sound pulse at the best frequency range, when possible with similar intensities. The signals were presented at 75-85 dB SPL.

One of the cells (AN_402) did not respond to the calling song (Fig 104 F) and another (AN_521) seemed to show a weak response by an increase of the spike activity (Fig. 104 E). These two interneurons were most sensitive to frequencies below 2 kHz, a frequency region where the calling song is not intense (cf. Fig. 10 A), and they had a high roll-off towards higher frequencies (Fig. 101 J,N).

One unidentified neuron seemed to increase its spike discharge both at increasing song intensity and especially after the dropping of the sound levels (Fig. 104 I), which is a common situation during the courtship characterized by a slow amplitude (and frequency) modulation.

Tonic responses to the calling song were also displayed by two other cells (Fig. 104 A,G). There was however a large difference in the spiking rate achieved by these cells and in their degree of adaptation. AN_401 (Fig. 104 A) adapted strongly and it did not seem to encode the smooth end of the sound sequence although an abrupt sound gap was clearly detected. In contrast, AN_400/500 (Fig 104 G) showed less adaptation and detected the smooth end of a sound sequence.

All three T-shaped cells (AN_502, Fig. 104 B; AN_507, Fig 104 C; AN_501, Fig. 104 D) were phasic-tonic, but they responded differently to the song play-back. Contrasting with the response to a pure tone at one of the best frequencies (4 kHz, 75 dB) which was phasic-tonic, AN_502 had a phasic response at 75 dB with a very short spike burst at the song onset, although some depolarization was maintained during the sound presentation (Fig. 104 B). At 85 dB the phasic burst of the beginning was followed by tonic spiking with an adaptation (not shown on Figure 104). AN_507 responded with a phasic burst followed by a tonic discharge with nearly no adaptation (Fig. 104 C). In AN_501 the phasic burst was not very clear (Fig. 104 D), although it was present at lower intensities with pure tone stimulation (see Fig. 101 A). Sound gaps were very well represented with a fast repolarization followed by a phasic discharge in AN_502 (Fig. 104 B) and AN_507 (Fig. 104 C). AN_501 (Fig. 104 D) was different from the other two cells of

this morphological class in the course of the depolarization at the onset of the song. While a gradual increase in sound amplitude is accompanied by an increase in the cells depolarization in AN_502 and AN_507, this was not the case in AN_501.

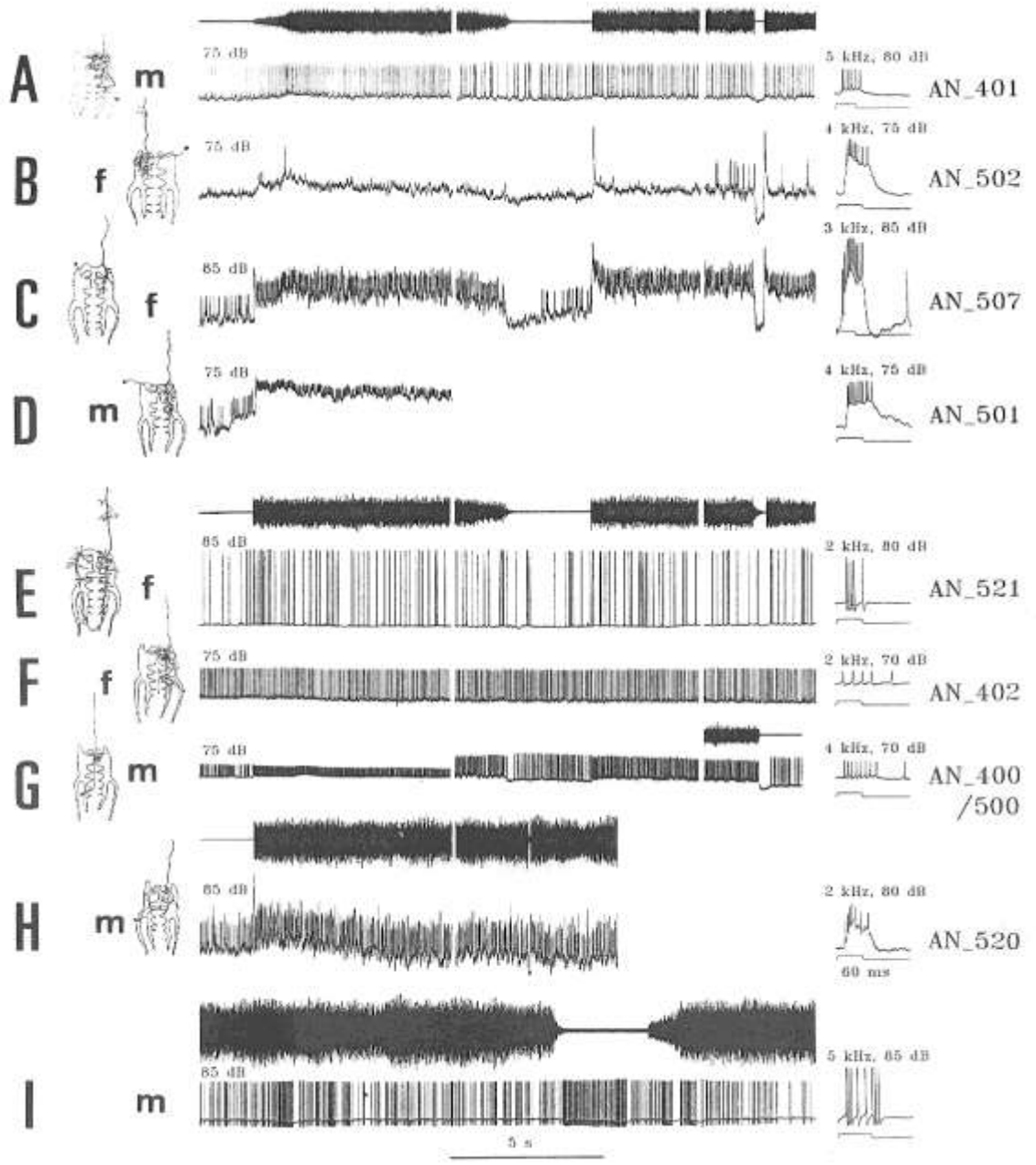


Figure 104 - Responses of auditory interneurons of *Cicada barbara lusitanica* to the conspecific calling song. Different neurons responding in different ways may take account for several particularities of the songs (see text for explanations). m male; f female.

The phasic-tonic response of AN_520 (Fig. 104 H) was very similar to the response displayed by AN_507 (Fig. 104 C), but the depolarization during the tonic discharge was much smaller.

The recorded interneurons of *C. barbara lusitanica* showed some of the necessary response properties that would be required by the nervous system to be able to recognize the song pattern (cf. Fig. 9). The slow increase in intensity found at beginning and at the end of the calling and courtship sequences, the amplitude variations characteristic of the courtship song, the abrupt beginning and end of the alarm and interaction sequences and the sudden sound gaps common on the alarm signals, are all encoded in different responses by different interneurons. Moreover, low frequency cells not responding to the calling song may play a role in the detection of other environmental noises, as those made by predators. However I did not examine or investigate what the masking caused by conspecific calling song would influence the ability of such cells to detect low frequency sound during song activity by other male cicadas.

7. Conclusion

The previous chapters have examined how cicadas may be able to solve the three principle tasks requested by a receiver in an acoustic communication system: 1) detection of the sound signal, 2) localization of the sender or the sound source, and 3) filtering and recognition of the signal.

1) Detection: The tympanic membranes of cicadas show a mechanical response to a wide range of sound frequencies, and these vibrations are picked up by the sensitive auditory organs. There is some degree of tuning exhibited by the auditory organ response as judged from auditory nerve recordings, and different auditory interneurons within the MAC show tuned responses as well. This allows the animals to increase the signal-to-noise ratio at certain

frequencies and therefore it improves their ability to detect certain sounds, since masking by other frequencies will be considerably reduced.

The different constructions of the body in male and female cicadas, which are subjected to different constraints (e.g. sound production in males and egg laying in females) might influence hearing sensitivity. From all species studied here, only *Tymp. gastrica* exhibited a marked difference of about 10 dB in the region of higher sensitivity in males as compared to females. This difference may be linked to the largest observed difference in the size of the tympana, and in the much larger air sacs of the males, found among the species studied here. In *Tib. quadrisignata*, on the other hand, females showed better sensitivities at higher frequences than the males but there is no obvious correlation with their morphology.

The several species analysed in this thesis all show the best hearing sensitivity in the same frequency range (3-5 kHz) irrespective of the differences in the spectral contents of their conspecific signals and in the morphology of their tympana. In particular, the apparent mismatch between sender and receiver for some cicada species presented here (Fig. 105) suggests that the animals may have some advantage by this low frequency tuning of their hearing system. A mismatch between the hearing threshold curves and the calling song spectrum was found in *Tett. argentata/atra*, *Tett. josei*, *Tib. quadrisignata* and *Tymp. gastrica*, species where the calling song is dominated by frequency components above 5 kHz. While in some cicadas with calling songs rich in low frequency components this discrepancy was not found (Enger et. al. 1969; Simmons et. al. 1971; Young and Hill 1977; Huber et. al. 1990), a similar discrepancy was reported in other cicadas by Popov (1981), Popov et. al. (1985), Popov and Sergeieva (1987) and Huber at al (1990).

Principally there appear to be two interpretations for this mismatch: a) There may be a strong selective force to detect low frequency sounds as they may be produced by a predator (Popov 1990b). The importance of hearing in the detection and localization of predators is well established for many insects (e.g. moths: Roeder 1962; lacewings: Miller 1984; crickets: Nolen and Hoy 1986; locusts: Robert 1989). Indirect support for an advantage of hearing low frequencies came from the observation that males and females show the same

tuning despite their obvious differences in the size of the tympana and in the acoustic input structures. The unmatch with the calling song may possibly also be used to detect the alarm signals in some species (e.g. *Tib. quadrisignata*) where the spectra of the alarm signal is shifted towards lower frequencies when compared to the calling

song. b) The mechanical design of the cicada ear may simply lock the auditory system in the low frequency range. In spite of the relatively broad frequency response shown by the tympanal vibrations (chapter 3), to this date too little is known about the mechanics and modes of vibration of the tympanal apodeme and the transduction properties of the auditory receptors for a conclusive statement.

However, it has to be kept in mind that the interpretation of an apparent mismatch to date is largely based on whole auditory nerve recordings. As shown before (chapter 4) there is a large number of auditory receptors, and some of which

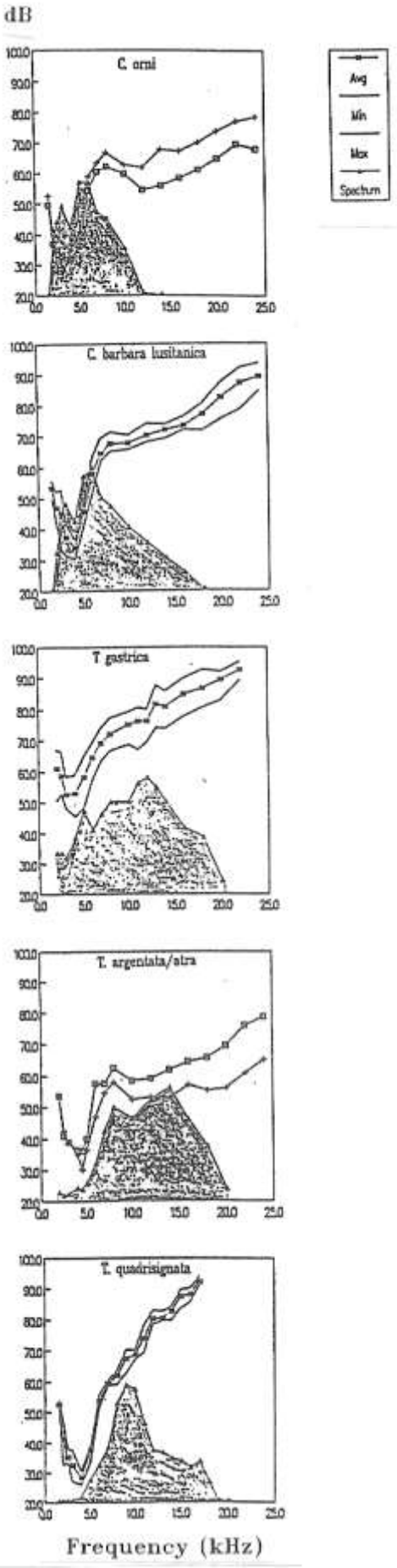


Figure 105 - Correspondence between the spectrum of the calling song and the auditory threshold of female cicadas. Thresholds were estimated by the earphone method with whole nerve recordings. The correspondence decreases from *Cicada* (A, B), where both curves match, to *Tibicina quadrisignata* (E), where a mismatch between the auditory threshold and the spectrum of the conspecific calling song is found.

may match the calling song spectra with their tuning but escape detection due to the method used. This question of a mismatch can be better approached by single cell recordings since, as was shown here in *C. barbara lusitanica*, there may be cells within the CNS that show a higher sensitivity to certain frequencies than would be expected from the hearing thresholds estimated with whole nerve recordings.

2) Localization of the sound source: The biophysical studies showed that the cicada ear is working as a pressure difference receiver and demonstrated that the main inputs are the tympana, and also the timbals in males and the third spiracles in females. Moreover, these investigations also demonstrated differences in the male and female hearing directionality which are related to morphological differences, such as the interaural peak occurring in males of *Tymp. gastrica* at 12-13 kHz, but not in females, and due to the sound input through the male timbals. Morphological data was crutial to support these findings since it showed that the several structures were acoustically connected. Important directional properties were found at several frequency ranges, namely at low frequencies, and this is likely to be important to the animal's behaviour.

The mechanical directional properties of the receiver found at certain frequencies are transduced by the auditory organ and can be found encoded in the auditory threshold curves at the same frequencies. These frequencies usually include the range of maximum hearing sensitivity and the regions corresponding to high energy in the spectra of the conspecific signals. Therefore, cicadas shall be able to solve the task of localization of a sound source with the information they can get from the ears. This data is also supported by observations on the behaviour of cicadas, since they can acoustically orientate themselves towards singing conspecifics in the field.

3) Recognition of a signal is obtained through processing of the sound signals by the nervous system.

The first processing step occurs, however, at the periphery, where filtering properties of the receiver (e.g. frequency response of the tympana, temporal properties of the receiving system,

capabilities of receptor neurons) may already narrow the signal's characteristics that will be further processed in the auditory pathway. In the cicadas studied in this thesis the summed responses of the receptor cells recorded in the auditory nerve copied the general time pattern of the songs but not the fast variations within a sound signal such as single sound pulses.

To look at the response of identified interneurons in the auditory pathway gives a much better image about what the nervous system obtains in information. In *C. barbara lusitanica* some auditory interneurons responded to several characteristics of the songs which may be necessary for recognition of the conspecific sound signals, e.g. raising and reduction in sound amplitude, adaptation or not to continuous sounds, responses to gaps in the song. However, here phonotactic experiments are urgently needed in order to determine which parameters in the male's song are indeed relevant and thus filtered by the nervous system of the female.

Another question deals with the ability of the cicada ear to discriminate frequencies. A signal may be frequency modulated and environmental sounds such as the ones made by predators may have a frequency spectrum quite different from the one exhibited by the conspecific songs. This problem has been enhanced in cicadas because the auditory organ is a compact bulb and is connected to the tympanum by a single apodeme. Doolan and Young (1981) found that the receptor cells within the auditory organ are orientated according to orthogonal axis. They suggested that this neuroanatomic feature could be a basis for frequency discrimination done by cells reacting differently to different modes of vibration in the tympana, the basis for frequency discrimination in the locust (Michelsen 1971b). Further studies of the morphology of the tympanic apodeme, which is not a simple solid stick, and of the connections of this structure with both the tympanic ridge and the auditory organ are needed, as well as a study on the possible modes of vibration of this structure. Unequivocal behavioural measurents for frequency discrimination are not available to date. However, since different interneurons are tuned to different frequencies, frequency discrimination is in principle possible. Clear behavioural experiments are needed to determine whether and which role this ability may play in the acoustic communication system of cicadas.

Final remarks

The investigations carried out in this thesis describe how the tasks imposed on the sender, -- i.e. a) to produce detectable and b) specific sound signals, and the receiver in an acoustical communication system, -- i.e. c) to detect the signals, d) to locate the sender, and e) to filter and recognise the message, are solved by the cicada male and female.

Several adaptations led to the improvment of the detectability and specificity of the sound signals generated by the male cicada. a) The morphology of the male reflects the constraints imposed by the need of sound production. Although presenting the same basical timbal mechanism, different species may use different solutions to enhance the detectability of their songs. At the strucural level different ways of achieving loudness are used by different species. These include, for instance, the use of a resonant cavity that allows the concentration of the energy in a certain frequency range (*Tib. quadrisignata*), or the use of different structures to radiate different frequencies of the calling song, as was shown here in *Tympanistalna gastrica*. Cicadas may show different behavioural adaptations that may improve their efficiency as a sound sender as well. Examples are the choice of the elevated calling sites or the use of different strategies by large and small cicadas in order to cover a broad area with their calling songs. b) In order to obtain the species specificity of their songs, cicadas exhibit several ways of improving the distinctiveness of their signals, at several levels. Different species may have timbals with different morphologies and mechanics generating a diverse number of sound pulses in each timbal cycle and different frequency spectra. This basic timbal cycle can be repeated in different time patterns which are governed by the central nervous system. The cicadas may add further variability to the signals by modulating them in amplitude and to some extent also in frequency. For this they may use the tensor muscle of the timbal or the abdomen.

c) The detection of the sound signals by the receiver is allowed by the sensitive ears of the cicadas. Auditory responses tuned to certain frequencies were measured in the summed activity of the whole auditory nerve as well as in identified interneurons within the metathoracic-abdominal ganglionic complex (MAC). This tuning leads to an improved signal-to-noise ratio by reducing the masking by other sounds with different frequency components. d) It was

demonstrated that the cicada ear works as a pressure-difference receiver, which allows discrimination of the direction of the sound signal at low frequencies. The acoustic inputs to the ear besides the tympana are sex specific and, correspondingly, sex specific differences in hearing directionality were found. Moreover, the directional characteristics measured in the vibrations of the tympanum were found encoded in the auditory nerve responses at the same frequencies. e) Some filtering properties of the ear, such as the frequency response of the tympanal vibrations and the transduction properties at the auditory organ, narrow the characteristics of the signals that can be used in further processing by the nervous system. The general time pattern of the songs was copied by the summed responses of the receptors recorded on the auditory nerve, but not the fast variations of the signals. Centrally, some acoustic interneurons were found whithin the MAC which responded to several characteristics of the songs that may be necessary for recognition of the intraspecific signals. Moreover, as different cells can exhibit different frequency tuning characteristics, frequency discrimination by cicadas is possible in principle.

Matching of the calling song of a sender by the auditory capabilities of the receiver is expected in an acoustic communication system. However, in some species studied in this thesis this matching did not seem to occur, an observation also reported from other species (e.g. see Popov 1990b). The mismach between the spectrum of the calling song and the hearing abilities of the receiver is most interesting since it likely indicates that strong selection pressures other than the detection of the conspecific calling song may have occurred. This problem is discussed and possible explanations are either the need for detection of predators and/or even the alarm signals produced by other cicadas. However, the interpretation of this apparent mismatch is largely based on the whole auditory nerve recordings, which measures the summed response of a very large number of receptor cells. A better approach to this problem is achieved by single cell recordings since there may be cells within the central nervous system that show a higher sensitivity to certain frequencies than would be expected from the hearing thresholds estimated with whole nerve recordings (e.g. *C. barbara lusitanica*).

Major findings in this thesis are a) in the mechanisms by which male cicadas can modulate the time-amplitude and frequency characteristics of their signals, using the tensor muscle of the timbal and the abdomen (chapter 5,6 of Sound production) in the biophysical basis for sound

radiation in *Tympanistalna gastrica*, where different structures are involved (chapter 7 of Sound production), and c) in the biophysical basis for hearing directionality studied in detail in *Tympanistalna gastrica* (chapter 3 of Sound reception). Other important findings concern the motor patterns governing the contraction of the timbal muscles during the calling song, and the auditory responses measured at the periphery and in some auditory interneurons.

I hope that throughout this work it became evident that it is crucial for the development of this area of research to stimulate an interdisciplinary approach, and that there is still a long way to go before we can understand the multiple aspects of sound communication in cicadas. I believe that some of the frontiers of our knowledge have been pushed forward in this respect, and the present findings may contribute to fertilize this field with new questions.

The morphology and anatomy of cicadas, which must have evolved submitted to the laws of the acoustical physics, are key factors in the study of the mechanisms responsible for sound production and radiation, as well as for sound reception and directional hearing. The coupling of the two systems in the males and the complexity of the biophysical processes involved make it a challenge to understand the underlying mechanisms, but, at the same time, turns this research into a most exciting one. Also the neuronal circuitry governing sound production and enabling the transduction, filtering and recognition of sound patterns is still an open area to the curiosity and skills of the researcher.

However, the complementary line of research should be the comprehension of the capabilities and constraints of the sound production and sound reception systems to the actual insect behaviour. This can only be achieved through patient and conscious field observations and well planned behavioural experiments. Devising such experiments with cicadas has proved very difficult, but this challenge only makes such a research even more needed and stimulating.

Acknowledgements

Many persons did contribute to this thesis in many different ways. To indicate them all is certainly difficult. Among them I would like to thank the family, relatives, friends and colleagues for their support.

To Prof. Dr. Campos Rosado (FCL, Lisbon), whom already left us, I would like to express my gratitude for his friendship and nice words in difficult times.

To Prof. Dr. Pedro Duarte Rodrigues (FCL, Lisbon), responsible for this dissertation, I am grateful for his continuous support and incitement.

I am deeply grateful to Prof. Dr. Drs. h.c. Franz Huber (MPIV-Seewiesen, Germany), my supervisor, and Prof. Dr. Axel Michelsen (Odense University, Denmark), essentially because they allowed that many of my ideas became a reality. They received me in their laboratories, criticise my work and made suggestions, teached many things and offered me research opportunities, and opened so many doors. I also thank the enjoyable moments spent together.

To Dr. Mathias Hennig (MPIV-Seewiesen and Humboldt University, Berlin, Germany) I thank for helpful discussions leading to significant improvment of the text, and our enjoyable times in the beer gardens.

To Prof. Dr. Andrej Popov (Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia) and Dr. Mathias Hennig (MPIV, Seewiesen, Germany) for our nice collaborations in the studies of the sound radiation in *Tympanistalna gastrica* and in the work on the role of the tensor muscle, respectively.

Essentially to Dr. Hans-Ulrich Kleindienst (MPIV, Seewiesen, Germany), but also to Drs. Ole Naesbye Larsen and Lee Miller (Odense University, Denmark), I thank their help with the single cell recordings.

Again to Dr. Ole Naesbye Larsen (Odense University, Denmark) and also to Dr. Georg Klump (Technical University of Munchen, Garching, Germany) I thank their help with the Laser vibrometer technique.

To Dr. Theo Weber (MPIV, Seewiesen, Germany) I thank his encouragement and advices on the EMG recordings.

To Dr. Geoffrey Horseman (Technical University of Munchen, Garching, Germany), Dr. Meta Virant-Doberlet (University of Ljubljana, Slovenija) and Dr. Anne Marie Surlykke (Odense University, Denmark) I thank their teaching on axonal fillings.

To Prof. Dr. João Sousa Lopes and Dr. Valtrudes Oliveira (FCL, Lisbon), Eng. Bent Bach Andersen (Odense University, Denmark) and Eng. Peter Heinecke (MPIV, Seewiesen, Germany), I thank their help with several electronic apparatus.

To Eng. Carlos Fafaiol (IST, Lisbon) I thank his help in calibrating the audio equipment used for sound stimulation of the cicadas in order to obtain the auditory thresholds.

To Eng. Conceição Duarte (ESAS, Santar,m) and Dr. Artur Serrano (FCL, Lisbon) I thank their help in collecting cicadas.

To Mrs. Cornelia Bock (MPIV, Seewiesen, Germany) I thank her work on EM cross sections of the auditory nerves.

To Mrs. Ingeborg Graunke (MPIV, Seewiesen, Germany) I thank her help with the drawings of the identified neurons in *Cicada barbara lusitanica*.

To many colleagues in Lisbon and elsewhere by so many discussions and stimulation.

Bruel and Kjaer Portugal loaned me equipment used to check the sound insulating chamber used in some of the experiments.

Financial support for this work was provided by the University of Lisbon, the Portuguese institutes for scientific research -- INIC and JNICT, the Portuguese and Danish Ministries of Education, the Portuguese Ministry of Foreign Affairs, the Calloust Gulbenkian Foundation (Portugal), the Danish Natural Sciences Research Council, and by grants to Prof. Dr. Axel Michelsen (Odense University, Denmark) and to Prof. Dr. Drs. h.c. Franz Huber (Max-Planck-Institut für Verhaltensphysiologie, Seewiesen, Germany).

References

Aidley DJ (1969) Sound production in a Brazilian cicada. *J exp Biol* 51:325-337

Alexander RD (1957) Sound production and associated behaviour in insects. *Ohio J Sci* 57:101-113

Alexander RD (1960) *Sound communication in Orthoptera and Cicadidae*. In, *Animal sounds and communication*, WE Lanyon and WN Tovolga (eds). American Institute of Biological Sciences, Washington, pp.38-92

Alexander RD (1967) Acoustical communication in arthropods. *Ann Rev Entomol* 12:495-526.

Alexander RD, Moore TE (1958) Studies on the acoustical behavior of seventeen-year cicadas (Homoptera: Cicadidae: Magicicada). *Ohio J Sci* 58:107-127

Alexander RD, Moore TE (1962) The evolutionary relationships of 17-year and 13-year cicadas, and three new species (Homoptera, Cicadidae, Magicicada). *Univ Mich Mus Zool Misc Publ* 121:1-59

Autrum H (1940) Das Richtungshoren von Locusta und Versuch einer Hortheorie fur Tympanalorgane der Locustidentyp. *Z vergl Physiol* 28:326-352

Autrum H (1975) *L'audition et l'ouie des insectes*. In, *Traitee de Zoologie*, PP Grasse (ed)

Bailey WJ (1990) *Acoustic Behaviour of Insects: An evolutionary perspective*. Chapman and Hall, London, New York, Tokyo, Melbourne, Madras, XV+225p.

Ball EE, Oldfield BP, Rudolph KM (1989) *Auditory organ structure, development, and function*. In, *Cricket Behavior and Neurobiology*, F Huber, TE Moore and W Loher (eds), Cornell University Press, Ithaca and London, pp.391-422

Banta ES (1960) Apple orchard decline. *Proc Ohio Hortic Soc*, 118:88-90

Beamer RH (1928) Studies on the biology of Kansas Cicadidae. *Kansas Univ Sci Bul* 18(2):155-263

Bennet-Clark HC (1970) The mechanism and efficiency of sound production in mole crickets. *J exp Biol* 52:619-652

Bennet-Clark HC (1971) Acoustics of insect song. *Nature* 234:255-259

Bennet-Clark HC (1975) Sound production in insects. *Sci Progr* 62(246):263-283

Bennet-Clark HC, Ewing AW (1967) Stimuli provided by courtship of male *Drosophila melanogaster*. *Nature* 215:669-671.

Bennet-Clark HC, 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 elektronenmikroskop Direktabb Oberfl* 20:209-214

Bodson L (1976) La stridulation des cigales. Poesie grecque et realit, entomologique. *Antiquit Classique* 45(1):75-94

Boulard M (1973) Un type nouveau d'appareil stridulant chez les cigales. *C R Acad Sc Paris*, (D), 277(15):1487-1489

Boulard M (1976) Un type nouveau d'appareil stridulant accessoire pour les Cicadoidea. R,vision de la classification supèrieure de la superfamille (Hom.). *J Nat Hist* 10(4):399-407

Boulard M (1982) Les cigales du Portugal, contribution à leur étude (Homoptera, Cicadidae). *Annls Soc ent Fr* (N.S.) 18(2):181-198

Boulard M (1986) Une singulière evolution morphologique: celle d'appareil stridulant sur les genitalia des males de Carineta. Description de cinq especes nouvelles (Hom. Tibicinidae). *Annls Soc ent Fr* (N.S.) 22(2):191-204

Boulard M (1988) Taxonomie et nomenclature supérieures des Cicadoidea. Histoire, problémes et solutions. *EPHE, Biol Evol Insectes* 1:1-90

Boulard M (1990) Contributions à l'entomologie générale et appliqué. 2. Cicadaires (Homoptères Auchenorhynques). Premiére partie: Cicadoidea. *EPHE, Biol Evol Insectes* 3:55-245

Boulard M (1992) Identit, et bio-,cologie de *Cicadatra atra* (Olivier, 1790), la Cigale noire, in Gallia Primordia Patria (Homoptera, Cicadoidea, Cicadidae). *EPHE, Biol Evol Insectes* 5:55-86

Boulard M, Quartau JA (1991) *Tettigetta septempulsata*, nouvelle cigale lusitanienne (Homoptera, Cicadoidea, Tibicinidae). *EPHE, Biol Evol Insectes* 4:49-56

Brown JJ, Chippendale GM (1973) Nature and fate of the nutrient reserves of the periodical (17 year) cicada. *J Insect Physiol* 19:607-614

Busnel RG, Pasquinelly F, Dumortier B (1955) La trémulation du corps et la transmission aux supports des vibrations en résultant comme moyen d'information à courte portée des Ephippigères male et femelle. *Bull Soc Zool France* (Paris) 80:18-22.

Claridge MF (1985) Acoustic signals in the Homoptera: Behavior, Taxonomy and Evolution. *Ann Rev Entomol* 30:297-317

Claridge MF, Wilson MR, Singhrao JS (1979) The songs and calling sites of two European cicadas. *Ecol Entomol* 4:225-229

Cox RT, Carlton CE (1988) Paleoclimatic influences in the evolution of periodical cicadas (Insecta: Homoptera: Cicadidae: Magicicada spp.). *Am Midl Nat* 120(1):183-193

Cumber RA (1952) Notes on the biology of *Melampsalta cruentata* Fabricius (Hemiptera-Homoptera: Cicadidae), with special reference to the nymphal stages. *Trans R Ent Soc Lond* 103(6):219-237

Davis NT (1982) Improved methods for cobalt filling and silver intensification of insect motor neurons. *Stain Technology* 57(4):239-244

Dean WRJ, Milton SJ (1991) Emergence and oviposition of *Quintillia cf. conspersa* Karsch (Homoptera: Cicadidae) in the southern Karoo, South Africa. *J ent Soc sth Afr* 54(2):111-119

Doolan JM (1981) Male spacing and the influence of female courtship behaviour in the bladder cicada, *Cystosoma saundersii* Westwood. *Behav Ecol Sociobiol* 9:269-276

Doolan JM, Young D (1981) The organization of the auditory organ of the bladder cicada, *Cystosoma saundersii*. *Phil Trans R Soc Lond B* 291:525-540

Doolan JM, Young D (1989) Relative importance of song parameters during flight phonotaxis and courtship in the bladder cicada *Cystosoma saundersii*. *J exp Biol* 141:113-131

Dugdale JS, Flemming CA (1969) Two New Zealand cicadas collected on Cook's endeavour voyage, with description of a new genus. *New Zealand J Sci* 12:929-957

Dunning DC, Byers JA, Zanger CD (1979) Courtship in two species of periodical cicadas *Magicicada septendecim* and *Magicicada cassini*. *Anim Behav* 27:1073-1090

Enger PS, Aidley DJ, Szabo T (1969) Sound reception in the Brazilian cicada *Fidicina rana* Walk. *J exp Biol* 51:339-345

Esch H, Huber F, Wohlers DW (1980) Primary auditory neurons in crickets: Physiology and central projections. *J Comp Physiol* 137:27-38

Ewing AW (1989) *Arthropod Bioacoustics: Neurobiology and Behaviour*. Edinburgh University Press, Edinburgh, X+260p.

Fabre JH (1923) *Souvenirs entomologiques. Etudes sur l'instinct et les moeurs des insectes*. 5 serie, pp. 229-308. Delagrave, Paris

Fletcher NH, Hill KG (1978) Acoustics of sound production and of hearing in the bladder cicada *Cystosoma saundersii* (Westwood) (Hom. Cicadidae). *J exp Biol* 72:43-55

Fonseca PJ (1988) *Sobre a produção de som nos insectos*. Department of Zoology, Lisbon Faculty of Sciences, 55p.

Fonseca PJ (1991) Characteristics of the acoustic signals in nine species of cicadas (Homoptera, Cicadidae). *Bioacoustics* 3:173-192

Fonseca PJ (1993) Directional hearing of a cicada: biophysical aspects. *J Comp Physiol* 172:767-774

Fonseca PJ, Palmeirim J, Andrade F (1989) Biologia e informática. *Vértice* 22:35-42

Forel A (1908) *The Senses of Insects*. English Trans. London

Fullard JH, Thomas DW (1981) Detection of certain African insectivorous bats by sympatric tympanate moths. *J Comp Physiol* 143:363-368

Gomez-Menor Ortega J (1957) *Monografia de Cicádidos (Homoptera) de Espana*. Academia de Madrid ed., 89p

Gray EG (1960) The fine structure of the insect ear. *Phil Trans B* 243:75-94

Gwynne DT (1987) Sex-biased predation and the risky mate-locating behaviour of male tick-tock cicadas (Homoptera: Cicadidae). *Anim Behav* 35:571-576

Hagiwara S (1955) Neuro-muscular mechanism of sound production in the cicada. *Physiol Comp Oecol* 4:142-153

Hagiwara S, Uchiyama H, Watanabe A (1954) The mechanism of sound production in certain cicadas with special reference to the myogenic rhythm in insect muscles. *Bull Tokyio med dent Univ* 1:113-124

Hagiwara S, Ogura K (1960) Analysis of songs of japanese cicadas. *J Ins Physiol* 5:259-263

Hamilton DW (1961) Periodical cicadas, Magicicada spp., as pests in apple orchards. *Proc Indiana Acad Sci* 71:116-121

Haskell PT (1974) Sound Production. In, *The Physiology of the Insecta 2nd ed*., M Rockstein (ed), Vol.II, Academic Press, New York, pp.353-410

Heath JE (1968) Thermal synchronization of emergence in periodical 17-year cicadas (Homoptera, Cicadidae, Magicicada). Am Midl Nat 80(2):440-448

Hennig RM, Weber T, Moore TE, Huber F, Kleindienst H-U, Popov AV (1994a) Function of the tensor muscle in the cicada Tibicen linnei. J exp Biol 187:33-44

Hennig RM, Weber T, Huber F, Kleindienst H-U, Moore TE, Popov AV (1994b) Auditory threshold change in singing cicadas. J exp Biol 187:45-55

Hogmire HW, Baugher TA, Crim VL, Walter SI (1990) Effects of control of periodical cicada (Homoptera: Cicadidae) oviposition injury on nonbearing apple trees. J Econ Entomol 83(6):2401-2404

Howard WJ (1937) Bird behavior as a result of emergence of seventeen year locusts. Wilson Bull 48:43-44

Huber F (1983) Neural correlates of orthopteran and cicada phonotaxis. In, Neuroethology and Behavioral Physiology: Roots and Growing Points, F Huber and H Markl (eds), Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, pp.108-135

Huber F, Wohlers DW, Moore TE (1980) Auditory nerve and interneurone responses to natural sounds in several species of cicadas. Physiol Entomol 5:25-45

Huber F, Moore TE, Loher W (eds) (1989) Cricket Behavior and Neurobiology. Cornell University Press, Ithaca and London, XIII+565p.

Huber F, Kleindienst H-U, Moore TE, Schildberger K, Weber T (1990) Acoustic communication in periodical cicadas: neuronal responses to songs of sympatric species. In: Sensory systems and communication in Arthropods, FG Gribakin, K Wiese and AV Popov (eds). Birkhauser Verlag, Basel Boston Berlin, pp 217-228

Hutchings M, Lewis B (1983) Insect sound and vibration receptors. In, Bioacoustics, a comparative approach, B Lewis (ed). Academic Press, London, New York, Paris, pp.181-205

Joermann G, Schneider H (1987) The songs of four species of cicada in Yugoslavia (Homoptera: Cicadidae). Zool Anz 219(5/6):283-286

Josephson RK, Halverson RC (1971) High frequency muscles used in sound production by a katydid. I. Organization of the motor system. Biol Bull mar biol Lab, Woods Hole 141:411-433

Josephson RK, Young D (1979) Body temperature and singing in the bladder cicada, Cystosoma saundersii. J exp Biol 80:69-81

Josephson RK, Young D (1981) Synchronous and asynchronous muscles in cicadas. *J exp Biol* 91:219-237

Josephson RK, Young D (1985) A synchronous insect muscle with an operating frequency greater than 500 Hz. *J exp Biol* 118:185-208

Kalmring K, Kaiser W, Otto C, Kuhne R (1985) *Coprocessing of vibratory and auditory information in the CNS of different tettigoniids and locusts*. In, *Acoustic and Vibrational Communication in Insects*, K Kalmring and N Elsner (eds), Paul Parey, Hamburg, pp.193-202

Karban R (1982) Experimental removal of 17-year cicada nymphs and growth of host apple trees. *J NY Ent Soc* 90:74-81

Karban R (1983) Induced responses of cherry trees to periodical cicada oviposition. *Oecologia* 59:226-231

Katsuki Y, Suga N (1960) Neural mechanism of hearing in insects. *J exp Biol* 37:279-290

Krohne DT, Couillard TJ, Riddle JC (1991) Population responses of *Peromyscus leucopus* and *Blarina brevicauda* to emergence of periodical cicadas. *Am Middl Nat* 126(2):317-321

Lakes-Harlan H, Heller K-G (1992) Urtrasound sensitive ears in flies - a novel tympanal organ. *Proc 8th International Meeting on Insect Sound and Vibration*, Pommersfelden, 24-27 Sept, pp.42

Leroy Y (1979) L'univers sonore animal. R“les et évolution de la communication acoustique. Gauthier-Villars, Paris, p.350

Lloyd M, Dybas HS (1966a) The periodical cicada problem. I.Population Ecology. *Evolution* 20:133-149

Lloyd M, Dybas HS (1966b) The periodical cicada problem. II. Evolution. *Evolution* 20:466-505

Lockley TC, Young OP (1986) *Phidippus audax* (Araneae, Salticidae) predation upon a cicada (*Tibicen* sp.)(Homoptera, Cicadidae). *J Arachnol* 14(3):393-394

Lyon RH (1973) Propagation of environmental noise. *Science* 179:1083-1090

Marlatt CL (1908) A successful seventeen-year breeding record for the periodical cicada. *Entomol Soc Washington* 9(1/4):16-18

Mason AC (1991) Hearing in a primitive ensiferan: the auditory system of *Cyphoderris monstrosa* (Orthoptera: Haglidae). *J Comp Physiol A* 168:351-363

Metcalf ZP (1963) *General catalogue of the Homoptera*. North Carolina State College, Raleigh, USA

Michel K (1975) Das Tympanalorgan von *Cicada orni* L.(Cicadina, Homoptera). Eine Licht- und Elektronenmikroskopische Untersuchung. *Zoomorphologie* 82:63-78

Michelsen A (1971a) The physiology of the locust ear. II. Frequency discrimination based upon resonances in the tympanum. *Z Vergl Physiologie* 71:63-101

Michelsen A (1971b) The physiology of the locust ear. III. Acoustical properties of the intact ear. *Z Vergl Physiologie* 71:102-128

Michelsen A (1982) *Laser techniques in studies of hearing*. In, *Biomedical Applications of Laser Light Scattering*, DB Sattelle, WI Lee and BR Ware (eds), Elsevier Medical press, pp.357-370

Michelsen A (1983) *Biophysical basis of sound communication*. In, *Bioacoustics, A Comparative Approach*, B Lewis (ed), Academic Press, London, pp.3-38

Michelsen A (1985) *Environmental aspects of sound communication in insects*. in *Acoustic and vibrational communication in insects*, K Kalmring and N Elsner (eds), Paul Parey, Berlin

Michelsen A, Nocke H (1974) Biophysical aspects of sound communication in insects. *Adv Insect Physiol* 10:247-296

Michelsen A, Larsen ON (1978) Biophysics of the ensiferan ear. 1. Tympanal vibrations in bushcrickets (Tettigoniidae) studied with laser vibrometry. *J Comp Physiol* 123:193-203

Michelsen A, Fink F, Gogala M, Traue D (1982) Plants as transmission channels for insect vibrational songs. *Behav Ecol Sociobiol* 11(4):269-281

Michelsen A, Larsen ON (1983) *Strategies for acoustic communication in complex environments*. In, *Neuroethology and Behavioral Physiology: Roots and Growing Points*, F Huber and H Markl (eds), Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, pp.321-331

Michelsen A, Larsen ON (1985) *Hearing and sound*. In, *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, GA Kerkut and LI Gilbert (eds). Pergamon Press, Oxford, New York, pp.495-556

Michelsen A, Towne WF, Kirchner WH, Kryger P (1987) The acoustic near field of a dancing honeybee. *J Comp Physiol A* 161:633-643.

Miller LA (1971) Physiological responses of green lacewings (*Chrysopa*, Neuroptera) to ultrasound. *J Insect Physiol* 17:491-506

Miller LA (1982) *The orientation and evasive behavior of insects to bat cries*. In, *Exogenous and endogenous influences on metabolic and neural control*, vol I, ADF Addink and N Spronk (eds). Pergamon Press, Oxford, pp.393-405

Miller LA (1984) *Hearing in green lacewings and their responses to the cries of bats*. In, *Biology of Chrysopidae*, M Canard, Y Semeria and TR New (eds). W Jung Publishers, The Hague, pp.134-149

Moiseff A, Pollack GS, Hoy RR (1978) Steering responses of flying crickets to sound and ultrasound: mate attraction and predator avoidance. *Proc natn Acad Sci USA* 75:4052-4056

Moore TE (1973) *Acoustical Communication in Insects*. In, *Introductory Entomology*, VJ Tripton (ed), Brigham Young Univ Press, Syllabus. Provo, Utah, pp.307-323

Moore TE, Sawyer RT (1966) The mechanism of cicada tymbal action (Insecta: Homoptera: Cicadidae). *Am Zool* 6:509

Moulds MS (1975) The song of the cicada *Lembeja brunneosa* (Homoptera: Cicadidae) with notes on the behaviour and distribution of the species. *J Aust Entomol Soc* 14:251-254

Myers JG (1928) The morphology of the Cicadidae. *Proc Zool Soc Lond* 25:365-472

Myers JG (1929) Insect Singers. A Natural History of the Cicadas. London, Routledge

Myers IH, Myers JG (1924) *The sound-organs of New Zealand Cicadidae*. Rep 16th Meeting Austr Assoc Advanc Sci, Wellington. pp.420-432

Myers JG, Myers IH (1928) The significance of cicada song. A problem of insect communication. *Psyche* 8:40-57

Nocke H (1971) Biophysik der Schallerzeugung durch die Vorderflugel der Grillen. *Z vergl Physiol* 74:272-314

Nolen TG, Hoy RR (1986) Phonotaxis in flying crickets. I. Attraction to the calling song and avoidance of bat-like ultrasound are discrete behaviors. *J Comp Physiol* 159:423-439

Ossiannilsson F (1949) Insect drummers. A study of the morphology and function of the sound-producing organs of Swedish Homoptera, Auchenorrhyncha. *Opusc Ent (Suppl)* 10:1-146

Patterson IJ, Cavallini P, Rolando A (1991) Density, range size and diet of the European jay *Garrulus glandarius* in the Maremma Natural Park, Tuscany, Italy, in summer and autumn. *Ornis Scand* 22:79-87

Pinedo C (1981) Consideraciones sobre la terminolog¡a empleada en la descripci¢n de los cantos de los insectos. *Graellsia* 37:123-137

Popov AV (1969) Comparative studies of the communication sound signals and some principles of the organization of the acoustical system in cicadas and Orthoptera. *Trudy vses ent obozr* 53:182-221

Popov AV (1971) *Synaptic transformation in the auditory system of insects*. In, *Sensory processes at the neuronal and behavioural level*. Academic Press, New York, pp 301-320

Popov AV (1975) The structure of the timbals and the characteristics of the sound signals in singing cicadas (Homoptera, Cicadidae) in the southern regions of the USSR. *Entomol Obozr* 54:258-291

Popov AV (1981) Sound production and hearing in the cicada, *Cicadetta sinuatipennis* Osh. (Homoptera, Cicadidae). *J Comp Physiol* 142:271-280

Popov AV (1989) Species of singing cicadas revealed on the basis of peculiarities of acoustic behaviour. 1. *Cicadatra cataphractica* Popov (ex. gr. *querula*) (Homoptera, Cicadidae). *Rev Entomol URSS* 68:291-307

Popov AV (1990a) Species of singing cicadas revealed on the basis of peculiarities of acoustic behaviour. 2.*Cicadetta petrophila* (ex. gr. *inserta*) (Homoptera, Cicadidae). *Rev Entomol URSS* 69:579-586

Popov AV (1990b) *Co-evolution of sound production and hearing in insects*. In: *Sensory systems and communication in Arthropods*, Gribakin FG, Wiese K, Popov AV (eds). Birkhauser Verlag, Basel Boston Berlin, pp 301-304

Popov AV, Shuvalov VF (1977) Phonotactic behaviour of crickets. *J Comp Physiol* 119:111-126

Popov AV, Aronov IB, Sergeieva MV (1985) Calling songs and hearing in cicadas from Soviet Central Asia. *Zh Evol Biokh Fiziol* 21:451-462

Popov AV, Sergeieva MV (1987) Sound signalization and hearing in the Baikal cicada, *Cicadetta yezoensis* (Homoptera, Cicadidae). *Zool J* 66:681-693

Popov AV, Aronov IB, Sergeieva MV (1991) Frequency characteristics of the tympanal organs and the spectra of communicative sound signals in cicadas (Homoptera, Cicadidae) from the Praskovejskaia valley. To the problem of matching between sound production and sound reception. *Rev Entomol URSS* 70:281-296

Pringle JWS (1954) A physiological analysis of cicada song. *J exp Biol* 31:525-560

Pringle JWS (1957) The structure and evolution of the organs of sound production in cicadas. *Proc Linn Soc Lond* 167:144-159

Prozesky-Schulze L, Prozesky OPM, Anderson F, Van der Merwe GJJ (1975) Use of a self-made sound baffle by a tree cricket. *Nature* 255(5504):142-143.

Quartau JA, Fonseca PJ (1988) An annotated check-list of the species of cicadas known to occur in Portugal (Homoptera: Cicadoidea). In *Proc 6th Auchen Meeting*, Turin, Italy, 7-11 Sept 1987, p.367-375

Reaumur RA (1741) *Mémoires pour servir à l'histoire des insectes*, 5. Paris

Reid KH (1971) Periodical cicada: mechanism of sound production. *Science* 172:949-951

Robert D (1989) The auditory behaviour of flying locusts. *J exp Biol* 147:279-301

Robert D, Hoy RR (1992) Fatal attraction: the sensory basis for acoustic parasitism in a tachinid fly. *Proc 8th International Meeting on Insect Sound and Vibration*, Pommersfelden, 24-27 Sept, pp.57

Robertson RM, Pearson KG (1983) Interneurons in the flight system of the locust: Distribution, connections, and resetting properties. *J Comp Neurol* 215:33-50

Roeder KD (1962) The behaviour of free flying moths in the presence of artificial ultrasonic pulses. *Anim Behav* 10:300-304

Roeder KD (1966) Acoustic sensitivity of the noctuid tympanic organ and its range for the cries of bats. *J Insect Physiol* 12:843-859

Roeder KD (1967) Turning tendency of moths exposed to ultrasound while in stationary flight. *J Insect Physiol* 13:873-888

Roeder KD, Treat AE (1961) The detection and evasion of bats by moths. *Am Sci* 49:135-148

Roeder KD, Payne RS (1965) Acoustic orientation of a moth in flight by means of two sense cells. *Symp Soc Exp Biol* 20:251-272

Sakai M, Yamaguchi T (1983) Differential staining of insect neurons with nickel and cobalt. *J Insect Physiol* 29(5):393-397

Shaw EAG (1974) *The external ear*. In, *Handbook of sensory physiology. V/1.Auditory system: Anatomy physiology (ear)*. WD Keidel and WD Heff (eds), Springer-Verlag, Berlin Heidelberg New York, pp 455-490

Simmons JA, Wever EG, Pylka JM (1971) Periodical cicada: Sound production and hearing. *Science* 171:212-213

Simmons PJ (1977) Neuronal generation of singing in a cicada. *Nature* 270:243-245

Simmons P, Young D (1978) The tymbal mechanism and song patterns of the bladder cicada, *Cystosoma saundersii*. *J exp Biol* 76:27-45

Simon C, Lloyd M (1982) Disjunct synchronic populations of 17-year periodical cicadas: Relicts or evidence of polyphyly? *J NY Entomol Soc* 90(4):275-301

Slansky F (Jr) (1980) Eating xylem not enough for longevity. *Bioscience* 30(4):220

Smith FF, Linderman RG (1974) Damage to ornamental trees and shrubs resulting from oviposition by periodical cicadas. *Env Entomol* 3:725-732

Smith KG, Wilkinson NC, Williams KS, Steward VB (1987) Predation by spiders on periodical cicadas (Homoptera, Magicicada). *J Arachnol* 15(2):277-279

Smith RL, Langley WM (1978) Cicada stress sound: an assay of its effectiveness as a predator defense mechanism. *Southwest Nat* 23(2): 187-196

Soper RS, Delyzer AJ, Smith LFR (1976a) The genus *Massospora* entomopathogenic for cicadas. Part II. Biology of Massospora levispora and its host Okanagana rimosa, with notes on *Massospora cicadina* on the periodical cicada. *Ann Entomol Soc Am* 69:89-95

Soper RS, Shewell GE, Tyrrell D (1976b) *Colcondamyia auditrix* nov. sp. (Diptera: Sarcophagidae), a parasite which is attracted by the mating song of its host, *Okanagana rimosa* (Homoptera: Cicadidae). *Can Entomol* 108:61-68

Spangler HG (1988) Hearing in tiger beetles (Cicindelidae). *Physiol Entomol* 13:447-452

Speare AT (1921) *Massospora cicadina* Peck, a fungous parasite of the periodical cicada. *Mycologia* 13:72-82

Stephen RO, Hartley JC (1991) The transmission of bush-cricket calls in natural environments. *J exp Biol* 155:227-244

Steward VB, Smith KG, Stephen, FM (1988) Red-winged blackbird predation on periodical cicadas (Cicadidae: *Magicicada* spp.): bird behavior and cicada responses. *Oecologia*

Strehl CE, White J (1986) Effects of superabundant food on breeding success and behavior of the red-winged blackbird. *Oecologia* 70:178-186

Vasvary LM (1966) Musculature and nervous system of the thorax, of the sound mechanism, and of a typical pregenetal abdominal segment of the male of the annual cicada, *Tibicen chloromera* (Walker) (Homoptera: Cicadidae). *NY Entomol Soc* 74:2-55

Vogel R (1923) Uber ein tympanales Sinnesorgan, das mutmassliche Hororgan der Singzikaden. *Z Anat Entwickl-Gesch* 67:190-231

Weber T, Moore TE, Huber F, Klein U (1988) Sound production in periodical cicadas (Homoptera: Cicadidae: *Magicicada septendecim*, *M. cassini*). In: *Proc 6th Auchen Meeting*, Turin, Italy, 7-11 Sept. 1987, pp.329-336

Wever EG, Vernon JA (1959) The auditory sensitivity of Orthoptera. *Proc Natl Acad Sci USA* 45:413-419

White J, Strehl CE (1978) Xylem feeding by periodical cicada nymphs on tree roots. *Ecol Entomol* 3:323-327

Wiegert RG (1964) Population energetics of meadow spittlebugs (*Philaenus spumarius* L.) as affected by migration and habitat. *Ecol Monogr* 34:217-241

Wiley RH, Richards DG (1978) Physical constraints on acoustic communication in the atmosphere: inplications for the evolution of animal vocalizations. *Behav Ecol Sociobiol* 3:69-94

Wilkinson RC, McClelland WT, Murillo RM, Ostmark EO (1967) Stridulation and behaviour in two southeastern Ips bark beetles (Coleoptera, Scolytidae). *Fl Entomol* 50(3):185-195

Wohlers D, Williams JLD, Huber F, Moore TE (1979) Central projections of fibers in the auditory and tensor nerves of cicadas (Homoptera: Cicadidae). *Cell Tissue Res* 203:35-51

Wohlers D, Bacon J (1980) Sexual dimorphism of motoneurons: Timbal muscle innervation in male periodical cicadas and homologous structures in females. *Cell Tissue Res* 209:371-382

Wolda H (1989) Seasonal cues in tropical organisms. Rainfall? Not necessarily! *Oecologia* 80:437-442

Wolff JO, Dueser RD, Berry, KS (1985) Food habits of sympatric *Peromyscus leucopus* and *Peromyscus maniculatus*. *J Mammal* 66:795-798

Yager DD, Hoy RR (1986) The cyclopean ear: a new sense for the praying mantis. *Science* 231: 727-729

Young D (1972) Neuromuscular mechanisms of sound production in Australian cicadas. *J Comp Physiol* 79:343-362

Young D (1973) Sound production in cicadas. *Aust Nat Hist* 17:375-380

Young D (1975) Chordotonal organs associated with the sound producing apparatus of cicadas (Insecta, Homoptera). *Z Morph Tiere* 81:111-135

Young D (1990) Do cicadas radiate sound through their ear-drums? *J exp Biol* 151:41-56

Young D, Hill KG (1977) Structure and function of the auditory system of the cicada, *Cystosoma saundersii*. *J Comp Physiol* 117:23-45

Young D, Josephson RK (1983a) Mechanisms of sound production and muscle contraction kinetics in cicadas. *J Comp Physiol* 152:183-195

Young D, Josephson RK (1983b) Pure-tone songs in cicadas with special reference to the genus *Magicicada*. *J Comp Physiol* 152:197-207