

Motor control and directional accuracy of phonotaxis in female field crickets

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November 2021

This thesis is submitted for the degree of Doctor of Philosophy

Preface

This dissertation is the result of my own work, except for the operation of the CT scanner and of the software used in the design of the 3D models of the muscles in Chapter 3. The CT scanner and the software were operated by Keturah Smithson, who is the CT scan technician of the Department of Zoology.

None of the work presented here is substantially the same as any that I have submitted, or, is being concurrently submitted for a degree, diploma or other qualification at the University of Cambridge or any other similar institution.

This dissertation does not exceed the prescribed word limit of 60,000 words.

Acknowledgements

I would like to thank Berthold Hedwig for his guidance, sharing his expertise with me, and his feedback during the writing of this dissertation. I would also like to thank all the members of the Neurobiology of Acoustic Communication Group for their support, particularly Steve Rogers, who was always willing to offer advice.

I sincerely thank Jesus College for funding me, and the Cambridge Philosophical Society and the Department of Zoology for their financial support towards the end of my PhD.

I also wish to thank Sonia Pascoal, Andrew Gillis, and Keturah Smithson for providing materials and technical expertise for the CT scans.

Finally, I wish to thank my friend Daria for her support during the stressful times while writing this dissertation.

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List of abbreviations

a	anterior
AN1	Ascending Neuron 1
AN2	Ascending Neuron 2
ANOVA	Analysis of Variance
Ba	Basalare
C.I.	Confidence Interval
CE	Compound Eye
CPG	Central Pattern Generator
CS	Cervical Sclerite
CT	Computed Tomography
Cx1, Cx2, Cx3	Prothoracic, mesothoracic, and metathoracic coxa
d	dorsal
Es	Esophagus
DN1	Descending Neuron 1
Ep	Episternum
Fl	Flagellum
H	Head
IID	Interaural Intensity Difference
IS1	first Intersegmental Sclerite
ITD	Interaural Time Difference
JM	Joint Membrane
l-r1CP	1 chirp left – 1 chirp right paradigm
l-r2CP	2 chirps left – 2 chirps right paradigm
La	labrum
Lb	labium
LN4	Local Neuron 4
Mx	maxilla
Msn	Mesonotum
Mst	Mesothorax
MstF	Mesothoracic Furca
Mtn	Metanotum
NM	Neck Membrane
ON	Omega Neuron
ON1	Omega Neuron 1
ON2	Omega Neuron 2
PBS	Phosphate-Buffered Saline
p	posterior
Pe	Pedicel
PF	Prothoracic Furca
PFA	Paraformaldehyde
Ph1 and Ph2	first and second Phragmata
PIA1, PIA2, and PIA3	first, second and third Pleural Arms
PIR2 and PIR3	second and third Pleural Ridges
Pn	Pronotum
PoR	Postoccipital Ridge
Pt	Prothorax
PtF	Prothoracic Furca

Sa	Subalare
SA1, SA2, and SA3	first, second, and third Sternal Apophyses
Sc	Scape
Spn1 and Spn2	external pits of first and second Spinae
SSP	Stop-Start Paradigm
St1 and St2	first and second Stigmata
Tg	Tegula
TN	T-shaped Neuron
TP	Tentorial Plate
v	ventral

Summary

This thesis addresses two aspects of the phonotactic behavior of female field crickets (*Gryllus bimaculatus*) as they orient towards singing males: the first one is how the auditory input is integrated into the motor activity underlying their walking responses, and the second one is how accurately they can localize a singing male in a dynamic stimulus situation. Although it has been established that the conspecific calling song is recognized via a circuit in the brain, it is not clear how pattern recognition is linked to descending motor control of phonotaxis. To analyze the auditory-induced motor responses, I recorded high-speed videos of crickets performing phonotaxis and tracked the movement of their bodies and appendages. The video analysis showed that when crickets commence phonotaxis, their body parts and appendages are activated and moved from anterior to posterior in the following order: antennae, head, prothorax, front legs, middle legs. During phonotaxis the antennae move continuously side-to-side in a rhythmic pattern, and on top of this rhythmic movement is superimposed a shift to the side the calling song is presented from. Moreover, the prothorax makes small rhythmic movements that are coupled to the stepping cycle, and on top of these rhythmic movements also steers towards the side the calling song is presented from.

Following up on the results of the video analysis, I recorded the activity of the antennal muscles of the scape in crickets that performed phonotaxis. The scape contains two muscles: the adductor muscle that adducts the antenna towards the median line, and the abductor muscle that abducts it laterally. The activity of the adductor muscle is coupled to the adduction movement of the antenna during the contralateral presentation of the calling song, while the activity of the abductor muscle is coupled to the abduction movement during the ipsilateral presentation of the calling song. The antennal movement and muscular activity – especially the abduction movement and the activity of the abductor muscle – are coupled to the calling song on a chirp-to-chirp basis. The neurites of the motoneurons of the antennal muscles are located in the deutocerebrum, while the ascending auditory pathway projects into the protocerebrum. I discuss that additional auditory brain interneurons must

be involved for the transfer and processing of the auditory-to-motor signal from the protocerebrum to the deutocerebrum.

I also investigated the function of several thoracic muscles for potential contribution to the prothoracic movements contributing to phonotaxis. Of all the muscles tested, only the activity of pronotal muscle 56 was coupled to the prothoracic movements in crickets performing phonotaxis. Specifically, the activity of muscle 56 was coupled both to the rhythmic prothoracic movements that are coupled to the stepping cycle and to the auditory-induced steering of the prothorax. Like the antennae, the prothorax turns to the active speaker and also responds to the calling song on a chirp-to-chirp basis. I discuss that auditory input to the motoneurons of muscle 56 in the prothoracic ganglion is likely indirect via a pathway descending from the brain.

Finally, I tested the accuracy of female crickets walking on a trackball as they performed phonotaxis towards a speaker oscillating constantly between 45° left and 45° right relative to their long axis. In a group of crickets, I used a drop of wax to fix the prothorax against the mesothorax and test the effect of the immobilization of the prothorax has on auditory steering. The performance of the crickets with the fixed prothorax was not statistically different from the performance of the crickets that could freely move the prothorax, however, the crickets with the fixed prothorax generally understeered towards the more lateral angles of stimulus. Overall, in this dynamic situation the angular resolution of the crickets was 6-11° in their frontal range, which is less accurate than the previously reported 1-2° for phonotaxis towards a static sound source. The results show that crickets find orientation towards a moving sound source more challenging than towards a static one. This was further corroborated with tests where the crickets steered to the correct side when two speakers positioned 5° to the left and 5° to the right alternated in the presentation of the calling song, meaning their angular resolution for static sound sources was at least 5°.

Chapter 1: General Introduction

A brief introduction to the evolution of hearing

Vertebrate and invertebrate organisms have evolved and maintained the sense of hearing to communicate and perceive sounds that potentially enhance survival, such as sounds made by predators. Fay and Popper (2000) put forward two views with regards to the evolution of hearing in vertebrates. The first one is that hearing has evolved as part of communication systems and is subject to environmental and anatomical constraints. According to this view, the evolution of the communication system of a species constitutes a unique case driven by the pressure to transmit the conspecific sounds with minimal distortion in its habitat; at the same time, the sounds that a species is capable of broadcasting and receiving are constrained by its anatomy and physiology. In the context of mate attraction, the hypothesis that habitats apply pressure to the properties of acoustic signals is based on the assumption that individuals that can attract potential mates from greater distances should have increased mating success (e.g. for anurans see Ryan, 1988).

The alternative view on the evolution of vertebrate hearing is based on the concept of auditory scene analysis, which refers to the ability to discriminate individual sound sources, when all of them are active at the same time (Bregman, 1990). Fay & Popper (2000) suggest that such an ability is of fundamental adaptive value, because all audible sounds are significant, in the sense that the fitness of a species would depend on its ability to segregate the relevant sound sources from the irrelevant ones. According to the auditory scene analysis theory, the factors that drive the development of auditory systems are the principle parameters of acoustic signals that make sound signals differ from one another. These principles are the same for all organisms, and even though auditory systems differ in terms of anatomy and physiology, they serve the same function: to discriminate different sound sources. In this case, hearing functions, such as frequency discrimination and sound source localization, would contribute to the listener's perception of the structure of an auditory scene (Fay and Popper, 2000).

Unlike hearing in vertebrates, which has a single evolutionary origin (Manley, 2012), hearing in insects has evolved independently at least 24 and possibly up to 29 times among seven orders (Coleoptera, Diptera, Hemiptera, Lepidoptera, Mantodea, Neuroptera, and Orthoptera) within the context of intraspecific communication, predator evasion, and, in the case of some parasitoid flies, host detection (Yager, 1999; Yack, 2004).

With respect to the sound receiving structures, insect ears are classified into pressure receivers and particle velocity receivers (Windmill and Jackson, 2016). Pressure receivers respond to changes in sound pressure. The biological solution for a pressure receiver in insects is the tympanal organ, which consists of a thin membrane, called tympanal membrane, backed by a cavity, which in the majority of the described tympanal organs is filled with air. There are however exceptions, where the tympanal membrane is backed not by air, but by fluid, such as in the ear of the green lacewing (Miller, 1970). Attached to the tympanal membrane – not necessarily directly – are scolopidial sensilla forming a chordotonal organ that is stimulated when the tympanal membrane oscillates in response to changes in sound pressure (Yack, 2004; Strauß and Lakes-Harlan, 2014). In the case of the bush-cricket ear, the tympanal membrane is backed by air, but the sensilla, which do not attach directly to the tympanal membrane, are inside a fluid-filled cavity (Sarria-S *et al.*, 2017; Celiker, Jonsson and Montealegre-Z, 2020)

In contrast to pressure-detecting tympanal organs, sensors that detect the particle velocity component of the sound need to be external hair-like or feather-like structures and project away from the body to sample the flow of the particles and be viscously dragged by it. In the case of the antennae, the sensory organ, termed Johnston's organ, is located in the second segment of the antennae of mosquitoes, midges, honeybees, and some drosophilid flies (Nadrowski *et al.*, 2011). Apart from audition, it can have other functions, such as graviception in fruit flies (Kamikouchi *et al.*, 2009) and mediation of flight control in moths (Sane *et al.*, 2007). The auditory organs of insects had generally been assumed to be "passive" oscillators, however, recent evidence suggest that active processes may

add energy to the oscillation, possibly to amplify weak signals and enhance sensitivity both in tympanal (Coro and Kössl, 1998; Kössl and Boyan, 1998; Windmill *et al.*, 2006; Mhatre and Robert, 2013; Mora *et al.*, 2015) and antennal auditory organs (Göpfert and Robert, 2001; Avitabile *et al.*, 2009).

Tympanal organs have apparently evolved from pre-existing mechanoreceptor organs (Meier and Reichert, 1990; Boyan, 1993; Fullard and Yack, 1993; Yager, 1999; Strauß and Lakes-Harlan, 2014), termed “precursor organs” (Yager, 1999; Strauß and Lakes-Harlan, 2014). The oldest fossil records of insect ears belong to Orthoptera and date back to the Paleogene, about 55-50 million years ago (Rust, Stumpner and Gottwald, 1999; Plotnick and Smith, 2012), and a fossil record of a stridulatory apparatus from a Jurassic katydid about 165 my ago indicates that conspecific acoustic communication had been established by then (Gu *et al.*, 2012). A fossil record of an ancestral bat species from the early Eocene (~52.5 my ago) has an ear morphology that suggests a lack of echolocation abilities (Simmons *et al.*, 2008), therefore hearing in Insecta possibly first evolved within the context of conspecific communication, and subsequently the hearing range expanded to include ultrasound as well (Strauß and Lakes-Harlan, 2014). The atympanate, vibrosensitive organs found in the tibia of cave crickets (Rhopidophoridae) could represent the ancestral state from which the tympanate ears of field crickets evolved (Jeram *et al.*, 1995; Strauß and Lakes-Harlan, 2014). Female field crickets, the study species of this thesis, possess a pair of ears on the tibia of their forelegs and perform positive phonotaxis towards singing males (Alexander, 1962; Ulagaraj and Walker, 1973; Hedwig, 2014) and negative phonotaxis away from the ultrasonic cries of bats, which prey upon crickets (Nolen and Hoy, 1984; Pollack and Martins, 2007).

Hearing in the context of conspecific communication

Acoustic communication is not merely the generation and detection of a signal; in the context of mate attraction, a signal transfers information regarding the signaler’s identity, capabilities, and motivation. This information can be encoded in properties of the acoustic signal, such as the carrier frequency and pulse rate, that will allow the receiver to choose a mate (for insects and anurans see Gerhardt & Huber,

2001). If the receiver prefers values that are centered around the population mean of a signal property, the preference is said to have a 'stabilizing' effect on the property; in contrast, preference for values at one end of the spectrum is said to have a 'directional' effect on the property (Wagner, 1998; Gerhardt and Huber, 2001). In general, call features used for species identification are under stabilizing selection, whereas features that are energetically expensive, such as the calling rate, are under directional selection (Ryan and Keddy-Hector, 1992; Gerhardt and Huber, 2001). By choosing an appropriate mate, the receiver can gain direct and/or indirect benefits. Direct benefits increase its fecundity i.e. quantity of offspring or survivorship, e.g. by selecting a healthy mate the chance of infection may be reduced (Gerhardt and Huber, 2001; Wagner, 2011), whereas indirect – genetic – benefits affect the quality of the offspring (Jia and Greenfield, 1997; Gerhardt and Huber, 2001).

Preference for certain signal properties may also be due to "sensory bias", meaning that the neural circuitry responsible for signal preference pre-existed acoustic communication and was subsequently utilized in that context (Ryan and Keddy-Hector, 1992). For example, some moth species use high frequency acoustic signals in the context of conspecific communication possibly because their auditory system was already tuned to the ultrasonic cries of bats for predator avoidance purposes (Nakano *et al.*, 2009).

Among insects, acoustic signaling is widespread in two groups of Orthoptera and one group of Hemiptera (Strauß and Lakes-Harlan, 2014; Greenfield, 2016). In most species that utilize acoustic communication, hearing is present in both sexes, but often only males are capable of acoustic signaling, and the females perform phonotaxis towards them. There are, however, a few species that form duets, in which case the most common behavior is for the males to track females replying to an initial call. A notable exception occurs in mosquitos, where the female's flight tone signals her presence before both sexes match their flight tones to form a courtship duet (Cator *et al.*, 2009; Robert, 2009). Duetting has been studied principally in the Orthoptera, particularly the tettigoniid subfamilies Ephippigerinae and Phaneropterinae, but also in the Plecoptera, Hemiptera, and

Neuroptera (review: Bailey, 2003). In phaneropterines, a duet is initiated by a distinctive call of the male, with the female replying within a species-specific time window, thus allowing for species recognition (Zimmermann, Rheinlaender and Robinson, 1989; Dobler, Heller and von Helversen, 1994). In species where the male call is long and/or complex, its conclusion is punctuated by a trigger pulse that cues the female to reply (Bailey and Field, 2000; Stumpner and Meyer, 2001). The female *Leptophyes punctatissima* replies to the male call with a delay of about 25 ms (Robinson, Rheinlaender and Hartley, 1986), which is one of the fastest auditory-to-motor responses in insects. Such a rapid response could be controlled at the level of the motor activity, with the auditory information bypassing the brain.

In field crickets, males generate a calling song to attract females by rhythmically rubbing a 'scraper' on the anal side of the left forewing against a 'file' on the lower side of the right forewing. Once a female is in close range, the male switches from calling to courtship song, and if the courtship is successful the female mounts the male (Alexander, 1961). Among taxa of crickets, there is a wide variety of conspecific songs with pulsing, trilling, or chirping patterns (Gerhardt and Huber, 2001).

In the case of the bispotted field cricket *Gryllus bimaculatus*, my study species, the males produce a calling song that consists of 3-6 pulses at 4-5 kHz of 20 ms duration and intervals of 20ms, grouped into chirps with a period of 2-3 Hz. Females exhibit a stabilizing preference for a pulse period of 34-42 ms (Doherty, 1985; Poulet and Hedwig, 2006), but other call properties likely contribute to the attractiveness of the signal as well (for *Acheta domesticus* see Stout et al., 1983; for *Teleogryllus commodus* see Bentsen et al., 2006; Brooks et al., 2005). The preference for a specific pulse rate allows for species identification (Ewing, 1989; Bailey, 1991), but it can also be indicative of young healthy males, as less- or non-attractive signals, such as ones with missing pulses, could be due to old age, disease, or injury (crickets: Ritchie et al., 1995; grasshoppers: Kriegbaum, 1989).

Neural processing of acoustic signals

The nervous system of insects is organized in units termed ganglia, with typically one ganglion found in each body segment, and the subesophageal and supraesophageal ganglia forming the brain in the head (Klowden, 2013). Adjacent ganglia are linked with a pair of longitudinal fiber tracts termed “connectives”, however, fusion between two or more ganglia can occur (e.g. Wigglesworth, 1959). Within the ganglia, specific regions have been associated with the processing of auditory information, and, among tympanate insects, the axons of auditory receptors project to equivalent tracts and association areas in the central nervous system, even when the tympanal ears are located in different segments of the body (Boyan, 1993).

Despite the variety in auditory organs among insect taxa, the neural processing of acoustic signals faces similar fundamental challenges. These challenges include frequency discrimination, sound source localization, pattern recognition, and dealing with self-generated signals (Hedwig & Stumpner, 2016). Auditory processing begins at the periphery, where the acoustic signal is encoded into spike activity by the primary auditory neurons. Auditory afferents integrate the energy of an acoustic stimulus over time, with integration times that decrease as the frequency of the acoustic stimulus increases. Using a leaky integrator model, Sabourin, Gottlieb and Pollack (2008) estimated the integration time of a prothoracic interneuron of the cricket *Teleogryllus oceanicus* at 18.6 ms for low frequency stimuli and 42 for high frequencies. Estimates of integration times differ among species and depend on the experimental method and integration model used; for instance, for the noctuid moth *Agrotis segetum* estimates of integration times range from 2 to 69 ms depending on the study (see Table 1 in Tougaard, 1998). The amplitude of the acoustic stimulus correlates positively with the spike rate of the neural activity and negatively with the response latency (Yager and Hoy, 1989; Imaizumi and Pollack, 2001). If the acoustic stimulus is long-lasting, the primary auditory neurons adapt and their firing rates decrease until they reach the steady state response after about 100 ms (Fullard, Forrest and Surlykke, 1998; Gollisch *et al.*, 2002). Adaptation allows the neuron to detect changes in the stimulus level, such as the onset of pulses in a conspecific song, thus facilitating the

recognition of the conspecific song pattern (Ronacher and Hennig, 2004). Non-acoustic mechanical stimuli, such as substrate vibrations, walking, or breathing, can also cause the tympanic membrane to vibrate, thus introducing noise into the auditory system (Schildberger and Hörner, 1988; Hedwig and Meyer, 1994).

In crickets, a tympanal ear is located on each front tibia. The posterior tympanal membrane is exposed to the external environment, while the other side of the membrane is backed by the main leg trachea (Larsen and Michelsen, 1978; Michelsen, 1998). The leg trachea functions as an acoustic trachea, it traverses the leg and has a wide opening to the lateral side of the thorax through an acoustic spiracle, thus allowing sound to enter and reach the internal side of the tympanal membrane. The auditory trachea crosses the prothorax, and, at the midline, connects acoustically with the trachea of the contralateral leg. The left and right auditory tracheas are separated by a membrane in the prothorax termed *medial septum*, which enhances the directional cues (Michelsen, 1998). Therefore, in theory, sound can act on the auditory system through four pathways: (i) directly from the outside, and internally through (ii) the ipsilateral acoustic spiracle, (iii) the contralateral acoustic spiracle, and (iv) the contralateral tympanal membrane (Michelsen, Popov and Lewis, 1994). However, non-invasive measurements with laser vibrometry showed that only the first three inputs contribute to the tympanal oscillations of the *G. bimaculatus* ear in response to the calling song frequency of 4.5 kHz, thus effectively making the cricket auditory system a three-input pressure-difference system (Michelsen, Popov and Lewis, 1994).

In *G. bimaculatus* each auditory organ has 45-60 afferents (Young and Ball, 1974) organized in a structure termed *crista acustica*, which is attached to the auditory trachea behind the tympanum (Larsen and Michelsen, 1978). The auditory afferents of bush-crickets and crickets are arranged tonotopically, meaning their frequency tunings are determined by their location in the *crista acustica* (bush-crickets: Oldfield, 1982, 1984; crickets: Oldfield, Kleindienst and Huber, 1986), similarly to the tonotopic organization of frequency sensitivity along the basilar membrane of the cochlea in the

vertebrate ear (Ehret, 1978). In crickets, the frequency tunings of the auditory afferents are clustered into three ranges: low-frequency (≤ 5.5 kHz), mid-frequency (10–12 kHz), and ultrasound (≥ 18 kHz), with around three quarters of the afferents falling into the low frequency range (Imaizumi and Pollack, 1999).

The afferents forward the auditory activity pattern to the prothorax, where a number of auditory interneurons have been identified (Wohlers and Huber, 1982; Atkins and Pollack, 1987; for a comparison of auditory processing among insects see Hennig, Franz and Stumpner, 2004). A pair of prothoracic mirror-symmetrical omega-shaped interneurons (ON1) have dendrites restricted to one side of the prothoracic ganglion, and their axonal projections overlap with the dendrites of the contralateral ON1 (Wohlers and Huber, 1982). Each ON1 receives excitatory input from the afferents ipsilateral to its dendrites and monosynaptically inhibits the contralateral ON1 (Selverston, Kleindienst and Huber, 1985). The ON1 that is activated with shorter latency and greater excitation inhibits the activity of the contralateral ON1 and as a result diminishes any concurrent inhibition. This mechanism of reciprocal inhibition enhances the binaural differences (see below) utilized in the localization of sound sources (Boyd and Lewis, 1983; Larsen, Kleindienst and Michelsen, 1989). Apart from crickets, bush-crickets also possess a pair of omega-shaped interneurons, termed ON, that enhance the binaural differences via reciprocal inhibition (Schul, 1997; Molina and Stumpner, 2005). In bush-crickets, the reciprocal inhibition mechanism of ON also helps with the discrimination of calling signals arriving simultaneously from different directions within a chorus (Römer and Krusch, 2000). Furthermore, ON suppresses the neural response to the calling signals that follow the leading signal, which results in female preference for the leading signal (Römer, Hedwig and Ott, 2002).

The number of auditory neurons ascending to the brain varies among insects. In crickets, there are two neurons that have axons ascending from the prothoracic ganglion to the brain. The first one, AN1, is tuned to the frequency of the calling song (4-5 kHz) and plays an important role in phonotaxis (Schildberger and Hörner, 1988). The second one, AN2, is tuned to ultrasound and triggers evasive

responses to bat-like sounds (Fullard, Ratcliffe and Guignion, 2005). Bush-crickets have more auditory interneurons ascending to the brain than field crickets; for instance, the duetting bush-cricket *Ancistrura nigrovittatan* has at least five ascending auditory interneurons, with one of them likely being homologous to AN1 (Stumpner, 1997) and two of them being homologous to AN2 (Stumpner, 1996), while AN5 is tuned to the female song frequency (Stumpner, 1999). Some insects have several more auditory interneurons ascending to the brain; for instance, at least fifteen ascending auditory interneurons have been described in cicadas (Huber, Wohlers and Moore, 1980; Fonseca, Münch and Hennig, 2000; Fonseca and Correia, 2007).

In crickets, apart from local and ascending auditory interneurons, there are also interneurons with descending axons (DN1-4) that forward the auditory information to more posterior ganglia, and T-shaped neurons that have both descending and ascending axons (Wohlers and Huber, 1982; Atkins and Pollack, 1987). In *Gryllus campestris*, DN1 can encode the temporal structure of the calling song, therefore this information descends to posterior ganglia, too (Wohlers and Huber, 1982). On the other hand, a T-shaped neuron (TN) exhibited only weak responses to auditory stimuli in the calling song range (Wohlers and Huber, 1982). A possibly homologous T-fiber in the bush-cricket *Neoconocephalus ensiger* is sensitive to ultrasound and likely functions as an ‘alarm’ for the presence of bats (Faure and Hoy, 2000b, 2000a)

Sound source localization

Sound is crucial for the survival and reproduction of many organisms, however its value would be limited if the receiving organism could not perceive where the sound is coming from. Whether a sound signifies the presence of a predator or a conspecific, its localization allows the receiving organism to respond most effectively. Therefore, the ability of sound source localization is one of the most fundamental aspects of an auditory system.

Despite the differences among the ears of different species, almost all of them utilize binaural cues to locate sound sources, though one exception may be the mantis with its “cyclopean” ear (Yager

and Hoy, 1986; Yack and Fullard, 1993). Specifically, the interaural time and intensity differences, abbreviated ITD and IID respectively, are used to locate a sound source in the horizontal plane. Turning towards a sound source decreases the binaural differences, whereas turning away increases them.

Due to their body size relative to the wavelength of a sound signal, binaural differences in insects are small. For example, the between-ears distance of a cricket is around 12 mm and the IID is just 1-2 dB at the calling song frequency (Michelsen, Popov and Lewis, 1994). In the parasitoid fly *Ormia ochracea*, which also localizes male crickets via their song, the ITD can be as low as 2 μ s and the IID is not measurable with a probe microphone because the wavelength is much greater than the interaural distance (Robert, Miles and Hoy, 1996).

Behavioral tests can provide estimates of the binaural cues that animals require to locate a sound source and how accurately they can orient towards it. Two types of behavioral tests have been used: closed-loop and open-loop. In a closed-loop test the animal can update the directional information because it is allowed to freely move relative to the sound source. One example of a closed-loop test is an arena test (Shen *et al.*, 2008), where the animal can freely turn and walk towards the sound source, therefore the sound pressure level it perceives changes with each movement. In an open-loop test, the animal is tethered to the sound field and receives consistent directional information. One example of an open-loop experiment is that of a cricket in “tethered flight”, where the position of its ears relative to the sound source is fixed, and the phonotactic steering is indicated by the bending of the abdomen (May, Brodfuehrer and Hoy, 1988; Wyttenbach and Hoy, 1997). Another example is a trackball test, where a cricket is tethered by the thorax and walks on a lightweight ball, while its position relative to the sound source is fixed (Hedwig and Poulet, 2005). Whenever the cricket walks, it causes the ball to rotate, and a sensor tracks its rotation and as a consequence the movement of the cricket (Hedwig and Poulet, 2005). The ability of an animal to localize a sound source is likely to appear more acute in the lab than in the field, because in the lab the background noise and the degradation of the acoustic stimulus are minimized, and as a result the

measured performance is more likely to be near its theoretical optimum. Nevertheless, the data collected in the lab can be used to form models of the directional processing and to postulate whether the comparison and processing of the binaural differences occurs in the periphery or the central auditory system, as discussed below.

In the past, closed-loop tests had suggested that crickets are not capable of accurate angle-dependent phonotaxis but, instead, mostly lateralize, meaning they can only discriminate whether a sound source is to their left or right. Female *G. bimaculatus* in a Y-maze were unable to make correct turns when the male song was presented within 15° off their length axis (Rheinlaender and Blätgen, 1982; Fig. 1.1A). In another study, *T. oceanicus* in an arena almost purely lateralized and their angle of orientation did not correlate with the angle of stimulation (Oldfield, 1980; Fig. 1.1B). However, later tests under open-loop conditions suggest more acute directional hearing than previously reported. *T. oceanicus* in tethered flight had an angular resolution of $\pm 11.25^\circ$ in its frontal range, an angular resolution of $\pm 45^\circ$ for sources at 90° to the side, and of $\pm 33.75^\circ$ at the rear (Wytttenbach and Hoy, 1997; Fig. 1.1C). They were also able to discriminate the elevation of sound sources, although with a lower resolution: $\pm 45^\circ$ at the front and rear and $\pm 90^\circ$ underneath them (Wytttenbach and Hoy, 1997). Trackball tests showed that female *G. bimaculatus* possess hyperacute directional sensitivity and turn correctly even when the calling song is presented from 1° off their length axis (Schöneich and Hedwig, 2010; Fig. 1.1D). Measurement of the tympanal oscillations with laser vibrometry and recordings of the activity of the afferents showed binaural differences of afferent activity corresponding to just 0.4 dB/° and 42 $\mu\text{s}/^\circ$ respectively in the frontal range (Schöneich and Hedwig, 2010). It is not yet clear however what binaural cues crickets use at the neural level to control their steering behaviour, as the latency and spike differences in the ascending pathway can be small (Lv, Zhang and Hedwig, 2020).

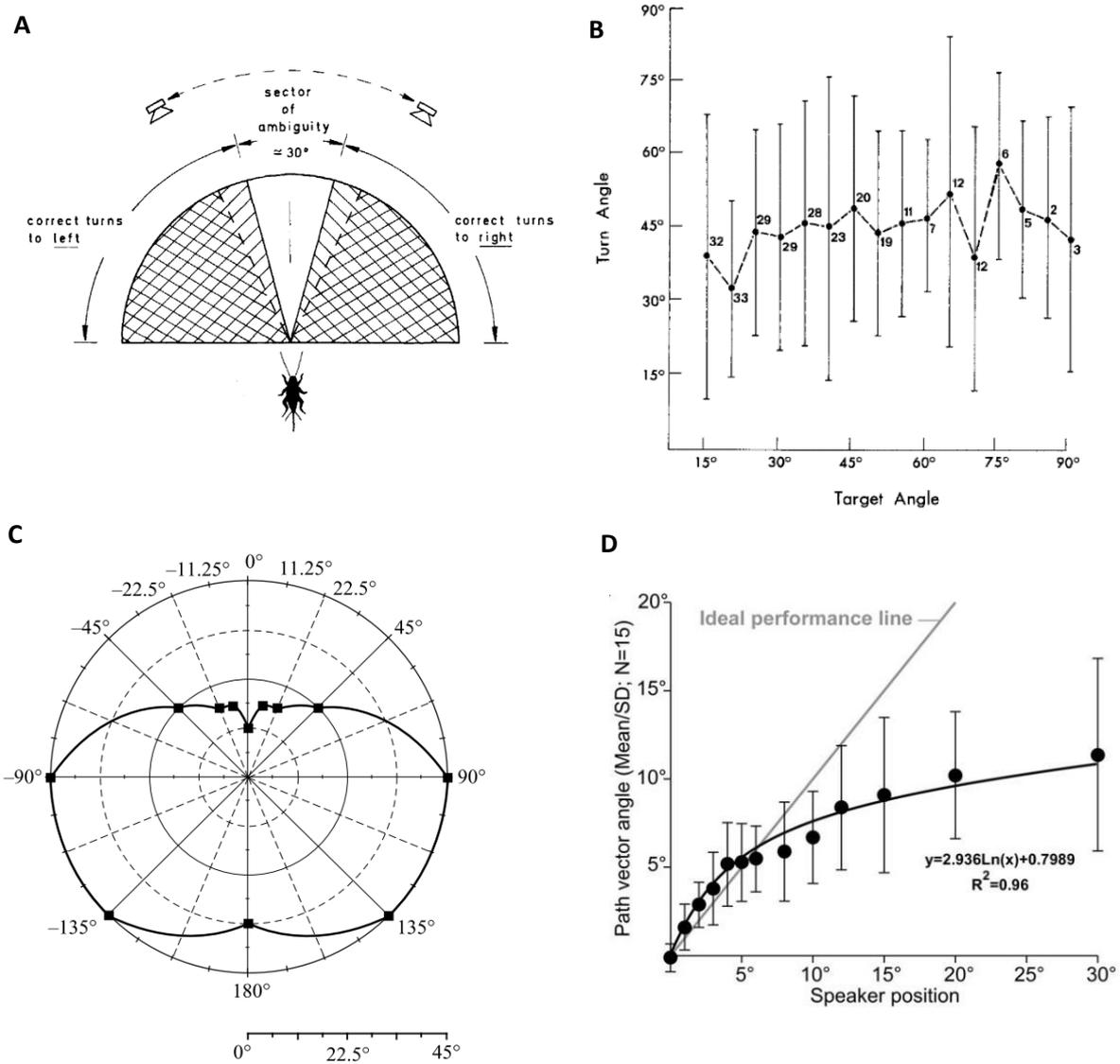


Fig. 1.1 Synopsis of results from studies on directional sensitivity of female field crickets during phonotaxis. **(A)** Y-maze tests with *G. bimaculatus*. Blank area indicates a sector of ambiguity when the male song is presented within 15° off the cricket's length axis; hatched areas show lateral sectors where the crickets showed significant left/right discrimination ($P < 0.05$); cross-hatched areas show areas where the crickets always made correct decisions. **(B)** Arena tests with *T. oceanicus* show no correlation between target angles and turn angles of the animals; error bars indicate standard deviations. **(C)** Angular resolution of *T. oceanicus* in tethered flight determined with a habituation–dishabituation paradigm. **(D)** Open-loop trackball tests with *G. bimaculatus* reveal hyperacute directional sensitivity in the frontal range of the crickets. **(A)** from Rheinlaender and Blätgen (1982); **(B)** from Oldfield (1980); **(C)** from Wytenbach and Hoy (1997); **(D)** from Schöneich and Hedwig (2010).

The only other insect with comparable levels of precision is the parasitoid fly *O. ochracea*, which locates its hosts, singing male crickets, by honing in on their songs with a precision of 2° (Mason,

Oshinsky and Hoy, 2001). Among Orthoptera, katydids are also capable of angle-dependent phonotaxis (Rheinlaender and Römer, 1990), albeit with a lower resolution of 6-10° in their frontal range, which corresponds to an IID of 1 dB (Rheinlaender, Shen and Römer, 2006). On the other hand, female *Chorthippus biguttulus* grasshoppers are capable only of lateralization and require minimum ITD and IID of 1 dB and 0.5-1 ms respectively to turn correctly to the side of the male song (Helversen and Rheinlaender, 1988).

The directional accuracy of *G. bimaculatus* and *O. ochracea* is comparable to that of some vertebrates. Males of the frog species *Odorrana tormota* are capable of localizing females within 1° (Shen *et al.*, 2008) and the barn owl has a localization error of less than 2° (Knudsen, Blasdel and Konishi, 1979). Elephants and humans have a directional acuity of 1° and 2° respectively (Heffner and Heffner, 1982; Fay, 1988), however, other mammals can be significantly less accurate: cattle have an angular resolution of 30° (Heffner and Heffner, 1992b) and naked mole rats are unable to localize brief sounds (Heffner and Heffner, 1992a). The species with the most acute directional hearing is the Atlantic bottle-nose porpoise (*Tursiops truncatus*), which exhibited an angular resolution of 0.7-0.8° to simulated echolocation clicks (Renaud and Popper, 1975).

Some insects have mechanisms for the enhancement of binaural cues. *O. ochracea* possesses ears that are mechanically coupled in such way that the time and intensity binaural cues of the mechanical response of the ears are significantly greater than those measured in the acoustic field (Robert, Miles and Hoy, 1996). Another example is found in the locust, where an unidentified interneuron in the metathoracic ganglion responds strongly with excitation to ipsilateral sound and is inhibited in response to contralateral sound, assumingly due to synaptic inhibition from contralateral interneurons (Römer, Rheinlaender and Dronse, 1981). This sound-direction-dependent inhibition mechanism increases binaural differences at the neuronal level and therefore potentially enhances the ability of the locust to localize a sound source. The omega neurons in the prothoracic ganglion of crickets and katydids use reciprocal inhibition to enhance binaural cues: the dendrites of an omega neuron are restricted on one side of the prothoracic ganglion and receive excitatory input from the

ipsilateral auditory afferents, while each omega receives inhibitory inputs at its dendrites via the axon of the contralateral omega neuron (crickets: Selverston et al., 1985; katydids: Molina and Stumpner, 2005; Römer and Krusch, 2000). As a consequence, the neurons respond with excitation when sound arrives from ipsilateral, i.e. at the side of their dendrites, and they are inhibited when sound arrives from the contralateral side. As the omega neuron ipsilateral to the sound source is activated with shorter latency and greater excitation, it inhibits the excitation and thereby the inhibitory output of the contralateral omega neuron (Selverston, Kleindienst and Huber, 1985).

In crickets, because the ear operates as a three-input (ipsilateral tympanum, ipsilateral spiracle, contralateral spiracle) pressure difference receiver at the frequency of the calling song (4.5 kHz), the force acting upon the tympanal membrane depends on the amplitude as well as the phase at which the sounds from the three inputs arrive (Michelsen, Popov and Lewis, 1994; Michelsen, 1998). Since the amplitudes and phases of the three inputs depend on the direction of the sound incidence, the directional response of the eardrum is “shaped” by the amplitudes and phases of the three inputs (Michelsen, Popov and Lewis, 1994). To enhance the directional cues, the medial septum, which is located in the midline and connects the left and right acoustic trachea, has been proposed to induce a phase shift upon the internal sound that passes through it from the contralateral side (Fletcher and Thwaites, 1979; Löhe and Kleindienst, 1994). The function of the medial septum as an “amplifier” of directional cues was supported by experiments in the lab with crickets that had their medial septa perforated and subsequently could not perform accurate phonotaxis (Wendler and Löhe, 1993); however, tests in the natural habitat showed that crickets with a perforated medial septum could still localize a speaker playing the calling song (Hirtenlehner, Römer and Schmidt, 2014). Nevertheless, perforation of the medial septum reduces the binaural time difference in the mechanical response of the tympanal membrane (Michelsen and Löhe, 1995), the afferent activity (Löhe and Kleindienst, 1994), and the activity of AN1 (Hirtenlehner, Römer and Schmidt, 2014)

Integration of sensory input into motor output

Sensorimotor integration is the integration of the sensory input into the motor response of an animal. It is a dynamic process, meaning that the sensory input potentially changes during the motor response and is updated before each integration “loop”. Sensorimotor control can be challenging to study because the motor output can depend on the context under which the sensory input is presented. This means that the same sensory input can result in different motor output depending on factors such as the posture of the animal, experience, and the motivational and behavioral state, e.g. whether the animal is hungry, active or standing still etc (Huston and Jayaraman, 2011). Insects make particularly attractive models for the study of sensorimotor integration because they can perform complex sensorimotor tasks, such as a hunting dragonfly adjusting its head orientation so that the image of the target prey remains centered on the “crosshairs” formed by the visual midline and the dorsal fovea of the compound eye (Olberg *et al.*, 2007). Due to their small brains and comparatively limited neural hardware, the neurons that are involved are often identifiable and physiologically accessible. For example, one such neuron is the descending contralateral movement detector that conveys visual information about impending collision from the brain to thoracic motor centers that trigger an escape response in the locust (Fotowat, Harrison and Gabbiani, 2011). Neurons with similar response and function have been found in pigeons (Sun and Frost, 1998), frogs (Nakagawa and Hongjian, 2010), fish (Preuss *et al.*, 2006), and fruit flies (Fotowat *et al.*, 2009).

With regards to the integration of the auditory input into motor responses in crickets, the animals exhibit specific responses to specific acoustic patterns. For example, the calling song induces positive phonotaxis, while the courtship song promotes assumption of the copulatory position by the female cricket (Alexander, 1962). Therefore, the auditory system first must recognize the acoustic pattern so that the animal can respond in a typical manner to the signal. Once pattern recognition has been established, in the case of positive phonotaxis two types of motor activity need to be generated: locomotion and steering via the integration of the binaural cues.

For an animal to recognize a behaviorally relevant sound pattern, the nervous system has to detect specific features in the neural activity that represents an acoustic stimulus. Several auditory neurons have been described that are involved in the extraction of features for the recognition of the calling song pattern by female crickets. In the prothorax, ON1 functions as a low pass filter that cuts off signals with pulse rates lower than that of the conspecific song (Nabatiyan *et al.*, 2003), and AN1 forwards to the brain only the sounds that feature the conspecific frequency of 4-5 kHz (Schildberger and Hörner, 1988). In the brain, a neural circuit functions as a coincidence detector for the detection of the pulse pattern of the calling song (Schöneich, Kostarakos and Hedwig, 2015). The coincidence detector selectively responds when the direct response representing the acoustic input coincides with a response that has been delayed by 30-40 ms, which is equal to the pulse period of the calling song (Schöneich, Kostarakos and Hedwig, 2015).

It is not yet clear how auditory pattern recognition is linked to descending motor control for phonotaxis. Hyperpolarization of the AN1 ipsilateral to the calling song to reduce its spike activity results in the animals steering contralaterally (Schildberger and Hörner, 1988). The hyperpolarization tests suggested that the binaural differences between the left and right AN1 play a role in directional processing by causing the animals to steer towards the side of the more excited AN1. However, recordings of AN1 activity in the brain showed that the bilateral latency differences of AN1 activity are small, and the spike differences are also small for stimuli presented in the frontal range of the animals, 1-3 spikes per chirp when the calling song is presented at 3° off the animals' length axis (Lv, Zhang and Hedwig, 2020). Since behavioural tests had shown that the animals were able to steer to the correct side even at small angles of stimulation (Schöneich and Hedwig, 2010; Lv, Zhang and Hedwig, 2020), it is not clear if these small binaural cues arriving at the brain are sufficient to control auditory steering.

Crickets steer towards individual pulses with a short latency of 55-60 ms (Hedwig and Poulet, 2004, 2005) and will steer towards non-attractive patterns inserted into the normal calling song, even though the non-attractive patterns are not sufficient to initiate phonotaxis when presented on their own (Poulet and Hedwig, 2005). This means that the pattern recognition circuit in the brain

(Schöneich, Kostarakos and Hedwig, 2015) is not constantly recognizing the calling song during phonotaxis. However, it is not known, if, once pattern recognition has established, auditory steering is controlled locally in the prothoracic ganglion or via a descending pathway from the brain. So far, no muscles or neurons involved directly in the auditory-to-motor integration have been identified. The projection sites of some auditory receptors indicate that their axons do not interact with the ascending pathway but may give input to DN1 that relays auditory information to posterior ganglia (Imaizumi and Pollack, 2005).

The left-right movement of the front legs that contributes to the auditory steering is superimposed on the stepping pattern (Baden and Hedwig, 2008; Petrou and Webb, 2012; Witney and Hedwig, 2011), it is therefore likely that the control of locomotion and auditory steering is decoupled. Even though the left-right movement of the front legs depends on the direction of the calling song, there is no 1:1 coupling of leg movement or tibia motoneuron activity to the pulses of the calling song (Baden and Hedwig, 2008), despite the ability of the animals to steer towards individual pulses (Hedwig and Poulet, 2005).

During phonotaxis crickets steer the prothorax to the sound direction (Witney and Hedwig, 2011; Petrou and Webb, 2012). To investigate the contribution of the prothoracic movements to auditory steering, I used a high-speed camera to track the lateral bending of the prothorax against the mesothorax in crickets performing phonotaxis on a trackball, and subsequently tested the functionality of muscles that potentially contribute to the prothoracic movements. Moreover, I tracked the lateral movements of the antennae and recorded the activity of the antennal abductor muscles (Honegger *et al.*, 1990) during phonotaxis. Even though it is known that the crickets constantly move their antennae to scan their surroundings when they walk (Horseman, Gebhardt and Honegger, 1997), the antennal movements and the activity of the antennal muscles in response to the calling song had not been investigated before.

Aims and structure of chapters

By analyzing the phonotactic behavior of crickets, the first aim of my PhD is to shed more light onto how the auditory information is integrated into walking motor activity during phonotaxis. In the 2nd chapter, I use high-speed video recordings to track the movements of the antennae, head, prothorax, front legs, and mid-legs during phonotaxis. Based on the results of the video analysis, which showed that the antennae and the prothorax exhibit auditory responses, I tested the function of antennal and thoracic muscles, and, where feasible, recorded the activity of these muscles during phonotaxis. The tests with the thoracic and antennal muscles are the subject of the 3rd chapter

The second aim of my PhD is to test the accuracy of phonotaxis under dynamic conditions, and for this purpose the calling song was presented from a continuously moving speaker, an experiment that has not been performed before. In addition, I tested the contribution of the prothoracic movements to phonotaxis by fixing the prothorax of some animals so that they could not bend it laterally against their mesothorax. The dynamic steering experiments are the subject of the 4th chapter.

Chapter 2: Tracking of body and appendages during phonotaxis using high-speed video recordings

Abstract

Female crickets, *Gryllus bimaculatus*, perform phonotaxis towards singing males, however it is not yet clear how the auditory input is integrated into the motor movements exhibited during phonotaxis. When crickets steer towards the direction of the calling song, the trajectories of the front and middle legs shift towards the direction of the acoustic stimulus, however the movement of the antennae, head, and prothorax has not been investigated. Here, to shed more light onto the auditory-to-motor integration, I used high-speed video recordings to track the movement of the body parts and appendages of crickets performing phonotaxis on a trackball. I examined how the movements of the body parts and appendages correlate with each other and what effect the presentation of the calling song has on them. The results showed that when initially motionless crickets commence phonotaxis, the first appendages to be activated – approximately 180 ms after the start of the calling song – are the antennae, with the head, prothorax, front legs, and middle legs following in this order. During phonotaxis crickets continuously move their antennae like a pendulum between the left and right side and superimposed on this movement is a DC shift to the side of the speaker presenting the calling song. The prothorax also exhibits an oscillatory left-right movement, which is coupled to the stepping cycle. Specifically, during each step the prothorax tilts away from the front leg that is in the swing phase and towards the front leg that is in the stance phase. Superimposed on this oscillatory movement is again a DC shift to the side of the calling song. The anteroposterior movement of the tarsi of the front and middle legs indicated the swing/stance phase of the stepping cycle and was independent of acoustic stimulation, whereas the left-right trajectories tilted to the side of acoustic stimulation.

Introduction

Acoustic communication in field crickets is one of the best studied examples of acoustic behavior in the animal kingdom. Females recognize the species-specific pattern of the male song and perform

phonotaxis towards the singing males. Phonotaxis has been studied using behavioral tests where female crickets walked either freely (Murphey and Zaretsky, 1972; Bailey and Thomson, 1977) or on a trackball (Wendler *et al.*, 1980; Schmitz, Scharstein and Wendler, 1982; Hedwig and Poulet, 2004, 2005; Poulet and Hedwig, 2005), neurophysiology (Kostarakos and Römer, 2010; Schöneich, Kostarakos and Hedwig, 2015), or a combination of behavior and neurophysiology (Schildberger and Hörner, 1988). Although it has been established that the auditory input ascends from the ears, which are located on the tibia of the front legs, to the brain, where the pattern recognition circuit is located (Schöneich, Kostarakos and Hedwig, 2015), it is not well understood how the auditory input is integrated into the locomotion and the auditory-induced steering that occur during phonotaxis. Studies have focused on the movement of the legs during phonotaxis (Bailey and Thomson, 1977; Baden and Hedwig, 2008; Witney and Hedwig, 2011; Petrou and Webb, 2012), and to a lesser extent on the movement of the body (Petrou and Webb, 2012). The up/down movement of the front legs is coupled to the stepping cycle and is independent of the sound direction, whereas the left/right movement depends on the sound direction (Baden and Hedwig, 2008). Still, in *Gryllus bimaculatus* the left/right movement of the front legs does not exhibit a 1:1 coupling with the sound pulses of the calling song (Baden and Hedwig, 2008), even though crickets steer towards individual pulses with a latency of just 55-60 ms (Hedwig and Poulet, 2004).

In contrast to the legs, the contribution of the prothorax – specifically, its lateral bending against the mesothorax – to phonotaxis or walking and steering in general has not been explored extensively. In crickets, the musculature of the prothorax and mesothorax contains a number of longitudinal, dorsoventral, and oblique muscles that can move the prothorax laterally and dorsoventrally as well as roll it against the mesothorax (Furukawa, Tomioka and Yamaguchi, 1983; Honegger *et al.*, 1984; also see Chapter 3). Petrou & Webb (2012) tracked the movement of the body in freely walking crickets and reported that the prothorax and mesothorax exhibit some roll during auditory steering. The lateral bending of the prothorax potentially contributes to the orientation of

the body in other insects as well; for example, praying mantises bend the prothorax to the direction of a prey when they orient towards it using visual cues (Yamawaki *et al.*, 2011).

Also, the significance of antennal movements during phonotaxis has not been thoroughly investigated. The antennae consist of two basal segments, the proximal scape and the distal pedicel, and of a long slender flagellum. The head capsule contains the muscles that move the antennae in the vertical plane via the head-scape joint, and the scape contains the muscles that move the antenna in the horizontal plane via the scape-pedicel joint (Honegger *et al.*, 1990; also see Chapter 3). The antennae are sensory organs that can perceive a variety of sensory modalities, including tactile from obstacles during walking (Staudacher, Gebhardt and Dürr, 2005; Ritzmann *et al.*, 2012), the airflow during flight (Roy Khurana and Sane, 2016), and olfactory from potential mates or host-plants (Angioy *et al.*, 2003). They can also have other functions, such as time-compensation in solar compass orientation (Merlin, Gegear and Reppert, 2009), signaling courtship (Ellis and Brimacombe, 1980), and flight stabilization (Sane *et al.*, 2007). The antennae also play a role in cricket phonotaxis: when a cricket walks, it constantly moves its antennae to scan its frontal surroundings (Horseman, Gebhardt and Honegger, 1997), and if an object touches the antennae during phonotaxis, the cricket ceases walking to explore the object, meaning the tactile stimulation of the antennae overrides phonotaxis (Haber Kern and Hedwig, 2016).

To gain a deeper understanding of phonotaxis motor control, the aim of this study is to use high speed video recordings to track the movement of the body and appendages of female crickets upon the initiation of phonotaxis and during phonotaxis. The moment when phonotaxis initiates is significant because it allows to determine which segment/appendage is activated first and with how much delay relative to the onset of sound. This can shed light onto how the auditory input is integrated into the motor output during phonotaxis.

Materials and methods

Crickets

Final instars of female *G. bimaculatus* were isolated from males to avoid mating and habituation to the calling song upon final moulting. The isolated females were kept individually in boxes with unlimited access to food and water on a 12h:12h L:D photocycle and at a temperature of 24-28 °C. Nine to fourteen days after a cricket's final moult, which is the period when the responsiveness to the calling song peaks (Sarmiento-Ponce, Rogers and Hedwig, 2021), the forewings of the animals were removed and a 3 cm insect pin was attached vertically with a mixture of bees wax and resin (1:1 ratio) on the first abdominal tergites. To support video tracking, white spots (approximately 1mm diameter) were painted (Edding 780 Marker Pen) on the flagellum of each antenna (approximately 2 cm from the scape), on the centre of the head and the pronotum, and on the tarsi of the front and middle legs.

Experimental set-up

Crickets were tethered in a normal walking posture on top of a Rohacell foam ball (diameter 5.65 cm and weight 3 g) by bending the pin 90° and inserting it into a pin holder (Fig. 3.1). The ball was fitted into a transparent acrylic half sphere with 24 small holes that allowed air from an air supply to pass and gently lift the ball, so that it rotated when the cricket walked. A high-speed camera (Genie HM640, DALSA, Canada) positioned behind the cricket was focused and recorded all white-marked body parts and appendages via a mirror positioned above the cricket at a 45° angle. Illumination was provided by four red LEDs, because red light is not visible to *G. bimaculatus* (Zufall, Schmitt and Menzel, 1989) and would therefore not disrupt phonotaxis. To elicit phonotactic walking, two active speakers (Sinus live, Neo13s, Conrad Electronics, Hirschau, Germany) 25 cm from the trackball and at a 45° angle relative to the cricket's median line played the acoustic stimulus. The output of the signal generator was also fed to two red laser diodes that pointed at the bottom left and right corners of the video frame respectively; they provided an optical signal synchronised with the acoustic stimuli so that their timing was indicated in the video recording.

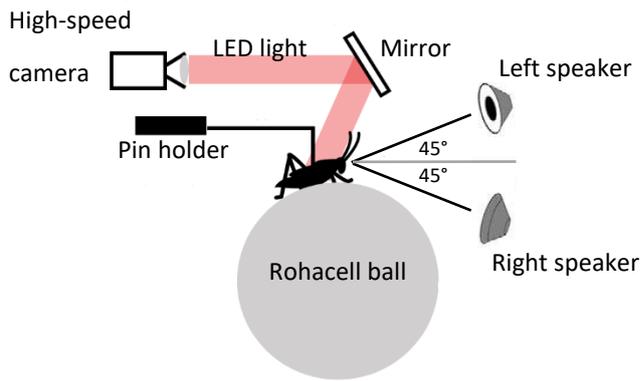


Fig. 2.1 Experimental set-up for the video recording of crickets performing phonotaxis.

Acoustic stimulus

A synthetic calling song was generated with Cool Edit 2000 (Syntrillium, Phoenix, USA, now Adobe Audition). The song consisted of chirps with a period of 500 ms, with each chirp consisting of 5 pulses at 4.8 kHz with a duration of 21 ms and a period of 42 ms. The pulses had a rising and falling ramp of 2 ms. The sound

intensity was calibrated at 75 dB SPL at the top of the trackball (Brüel and Kjær Nærum, Denmark, amplifier type 2610, microphone type 4191). The acoustic stimulus consisted of two paradigms: (i) the two chirps left – two chirps right paradigm (abbreviated l-r2CP) repeated 60 times, and (ii) the *stop-start* paradigm (abbreviated SSP), which consisted of 10 s silence, followed by 20 chirps presented from the left, repeated 20 times (Fig. 2.2). The aim of the first paradigm was to investigate the effect of the sound direction on the movement of the tracked body parts and appendages. The

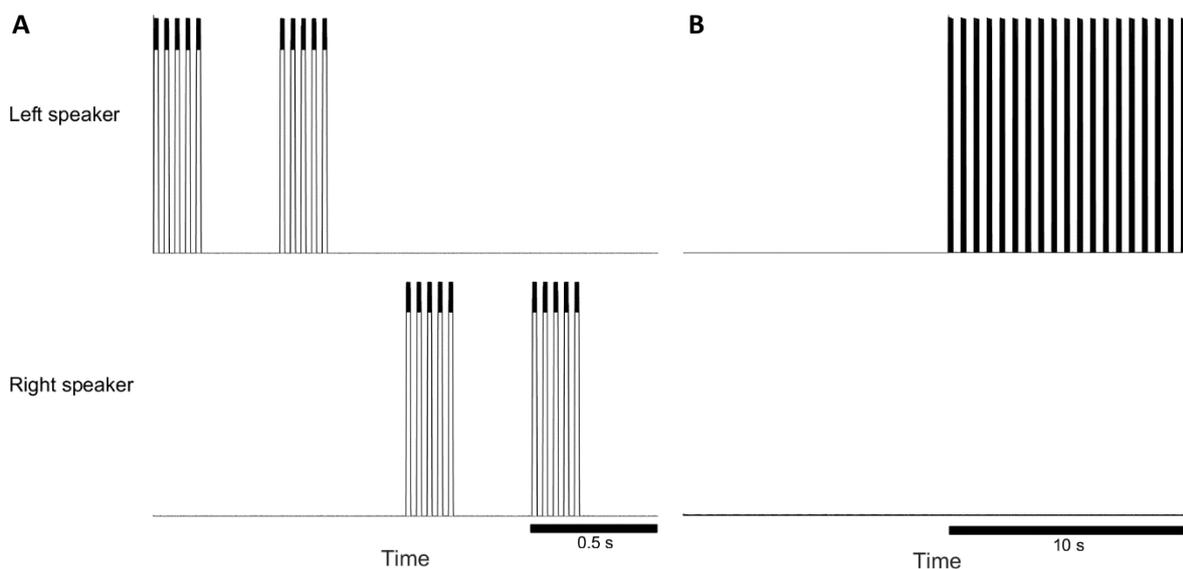


Fig. 2.2 The two paradigms of the acoustic stimulus: (A) two chirps left – two chirps right and (B) stop-start. Each chirp consists of 5 pulses at 4.8 kHz with duration 21 ms and period 42 ms. Each paradigm is shown for one repetition only.

aim of the second paradigm was to determine the delay in the activation of each body part and appendage relative to the sound when the cricket initiates phonotaxis.

Video recording and analysis

The control of the camera and the recording and saving of the videos to an external hard drive was done with the software Common Vision Blox (Stemmer Imaging Ltd, Surrey, UK). The videos have a frame rate of 300 fps, which corresponds to a temporal resolution of 3.33 ms, and the frame resolution is 480X640 pixels.

In each video I identified parts where the cricket responds to the two paradigms of the acoustic stimulus. To identify responses to the l-r2CP, I searched for parts of the video where the cricket walked consistently and steered towards the side of the active speaker. For responses to the SSP, I selected parts of the video where the cricket stopped during the silent intervals of the paradigm, then initiated phonotaxis when the calling song started again. The identified parts of the video where the cricket is responsive to the sound stimulus were edited and saved individually with the video-editing software PFV 4.0 (Photron Europe Limited, West Wycombe, UK). The edited videos have a duration of 2 s (600 frames) for the l-r2CP and 0.5 s (150 frames) for the SSP.

The videos recordings were imported into the software Tracker (Open Source Physics) to track the movements of the white-spotted body parts and appendages of the cricket. For each frame, the positions of the antennae, head, prothorax, tarsi of the front legs, and tarsi of the middle legs were determined by their coordinates on a two-axis coordinate system originating at the attachment point of the pin, with one axis running along the median line of the cricket (Fig. 2.3). For the movements of the antenna and the prothorax in response to the l-r2CP, I also determined their position angles (see Fig. 2.3 for an example of the position angle of the right antenna). In the analysis of the responses to the SSP, I used the position angle of each tracked body part and appendage to detect the moment when movement was initiated in response to the calling song, because the position angle detects changes both in the anteroposterior and lateral movement components.

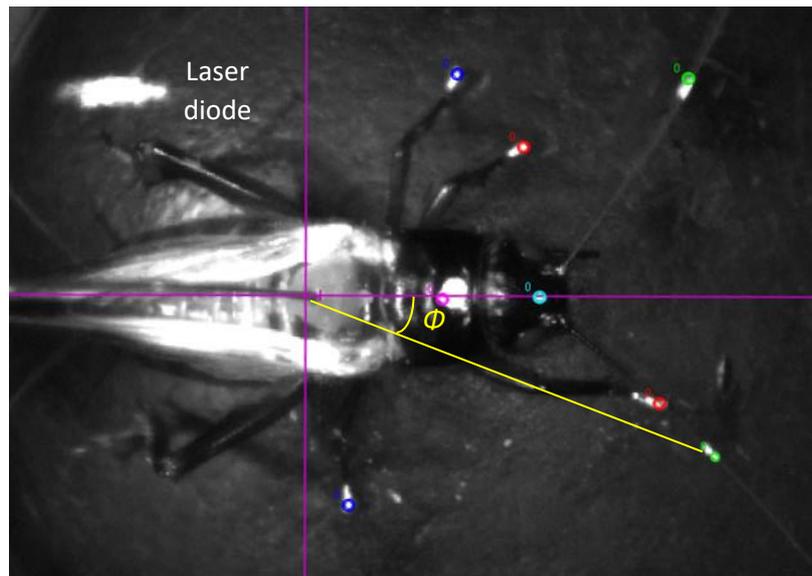


Fig. 2.3 Video frame demonstrating the tracking of the white spots on the antennae, head, prothorax, tarsi of the front legs, and tarsi of the middle legs. The position of each appendage and body part was determined with a two-axis coordinate system originating at the attachment point of the pin. Also shown the angular position of the right antenna. The white marker at the top left of the frame is generated by a laser diode and indicates the onset of sound from the left speaker.

The statistical analysis was performed in MATLAB R2019b (Mathworks, UK). I used paired sample *t*-test to test if the sound direction (left or right) had a significant effect on the position of the front and middle tarsi and on the position angle of the prothorax. I used two-way ANOVA to test if the properties that describe the movement of the antennae (scanning cycle duration, angular range, position angle) differ significantly between the left and right antenna and if they are affected significantly by the sound direction. Two-way ANOVA was also used to test if there is a significant difference among the activation delays of the body parts and appendages when motionless crickets commence walking and if the activation delays in response to the calling song differ from the activation delays when walking commences spontaneously. The statistical test used in each case is indicated both in the main text and the figure legends. For all statistical tests used, a *p*-value smaller than 0.05 was considered significant.

Results

Response to the l-r2CP

In this section I present an overview of the responses of the body parts and appendages to the l-r2CP, before analyzing in detail the responses of the antennae and the prothorax in the next two sections. The movement of the legs has been analyzed in detail elsewhere (Witney and Hedwig, 2011), so I conducted only a brief analysis of their movements here.

Out of 20 crickets tested, 8 performed phonotaxis (40%) when presented with the acoustic stimulus. During the l-r2CP, the crickets moved their antennae constantly side-to-side and independently of the acoustic stimulus; superimposed on these scanning movements of both antennae was a bias towards the side of the active speaker (Fig. 2.4A right). Specifically, the antenna ipsilateral to the active speaker exhibited a lateral bias, and the contralateral antenna exhibited a bias towards the median line of the cricket. The left-right movements of the antennae are moderately positively correlated with each other with a correlation coefficient of 0.52 ± 0.12 (mean \pm SD, $n = 8$ crickets, $N = 3$ repetitions of the l-r2CP per cricket). This means that both antennae are responding to the stimulus paradigm and are moving either leftwards or rightwards. On the other hand, the anteroposterior movements of the antennae are moderately negatively correlated with each other, with a correlation coefficient of -0.48 ± 0.11 . This means that when one antenna is moving towards the anterior the other is moving towards the posterior. In addition, the antenna ipsilateral to the active speaker exhibited a bias towards the posterior, whereas the contralateral antenna a bias towards the anterior (Fig. 2.4A left). Overall, the positive correlation of the lateral movements and the negative correlation of the anteroposterior movements indicate that when the antenna ipsilateral to the active speaker is abducted laterally, the other is adducted towards the median line.

The lateral movement of the head (Fig. 2.4B right) is observably coupled to the movement of the prothorax (Fig. 2.4C right). The correlation coefficient between the two is 0.62 ± 0.08 . The prothorax exhibited lateral movements, which are coupled to the stepping cycle, and superimposed

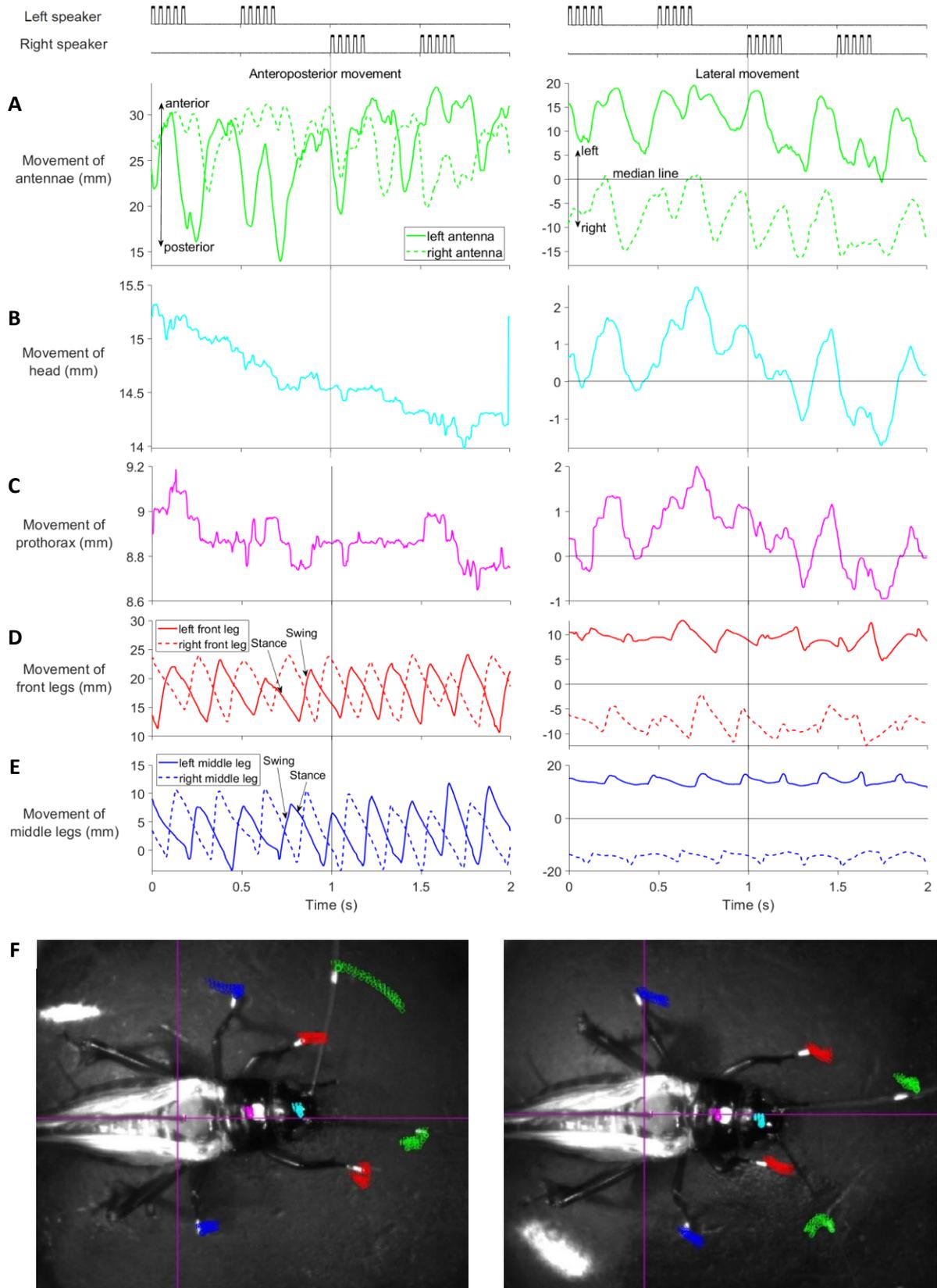


Fig. 2.4 Cricket responding to the l-r2CP. Tracked anteroposterior and lateral coordinates of (A) antennae, (B) head, (C) prothorax, (D) tarsi of the front legs, and (E) tarsi of the middle legs. (F) Frames showing auditory steering towards the left and right speaker. The colored paths show the movement of the tracked body parts and appendages over the previous 15 frames/50 ms (green: antennae; cyan: head; magenta: prothorax; red: front tarsi; blue: middle tarsi).

on these movements is a bias to the side of the active speaker. Since the head and the prothorax revolve within their joints, they exhibit some anteroposterior movement too (Fig. 2.4B,C left).

The forward-backward movement of the tarsi of the front legs is coupled to the stepping cycle (Fig. 2.4D left). The front tarsi move forward during the swing phase (posterior extreme position to anterior extreme position) of the stepping cycle and contact the Rohacell ball and rotate it backwards during the stance phase (anterior extreme position to posterior extreme position). The swing and stance phase last 108 ± 28 ms and 238 ± 44 ms respectively (mean \pm SD, $n = 8$ crickets, $N = 10$ stepping cycles per cricket). When one front leg is swinging, the other is generally in stance; the forward-backward movements of the front tarsi have a negative correlation coefficient of -0.82 ± 0.06 . The left-right movements of both front tarsi (Fig. 2.4D right) show a slight bias towards the active speaker but are not as strictly correlated as the forward-backward movements, with a negative correlation coefficient of -0.29 ± 0.04 . On average, the lateral position of the left front tarsus when the sound is presented from the left is 7.9 ± 1.7 mm, while when the sound is presented from the right the lateral position is 6.1 ± 1.4 mm, with the two values differing significantly (paired sample t -test, $P < 0.05$, $n = 8$ crickets, $N = 3$ repetitions of the l-r2CP per cricket). The corresponding values for the right front tarsus are -5.8 ± 1.2 mm and -7.5 ± 1.9 mm, and they also differ significantly (paired sample t -test, $P < 0.05$).

Similarly to the front tarsi, the forward-backward movements of the tarsi of the middle legs are coupled to the stepping cycle (Fig. 2.4E left) and have a negative correlation coefficient of -0.71 ± 0.07 . The left-right movements of the middle tarsi are uncorrelated (Fig. 2.4E right), with a correlation coefficient of 0.05 ± 0.11 . The lateral position of the left middle tarsus when sound is presented from the left is 16.1 ± 3.3 mm, while when sound is presented from the right, the lateral position is 14.8 ± 2.8 mm, with the two values not differing significantly (paired sample t -test, $P = 0.184$). The corresponding values for the right middle tarsus are -15.3 ± 2.7 mm and -16.5 ± 2.9 mm, and they also do not differ significantly (paired sample t -test, $P = 0.216$).

The left-right movements of the front and middle tarsi are correlated (Table 2.1). In general, when a front leg is in the swing phase, the ipsilateral middle leg is in the stance phase and vice versa (for a detailed analysis of the kinematics of the legs see Witney and Hedwig, 2011).

Table 2.1 Correlation coefficients (mean \pm SD, $n = 8$ crickets, $N = 3$ repetitions of the l-r2CP per cricket) for the left-right movement of the tarsi of the front and middle legs

		Middle tarsi	
		Left	Right
Front tarsi	Left	-0.68 \pm 0.05	0.67 \pm 0.04
	Right	0.67 \pm 0.07	-0.63 \pm 0.04

Movement of antennae during the l-r2CP

During the l-r2CP, the antenna ipsilateral to the active speaker moved constantly outwards (abduction) up to 40-55° laterally and the antenna contralateral to the active speaker inwards (adduction) up to slightly past the median line (Fig. 2.5). The correlation coefficient of the position angles of the two antennae is 0.54 \pm 0.11 (mean \pm SD, $n = 8$ crickets, $N = 3$ repetitions of the l-r2CP) and indicates, like the correlation coefficients of their lateral and anteroposterior movement components also indicated above, that their movements are moderately positively correlated with each other, and generally when one antenna is abducted the other is adducted.

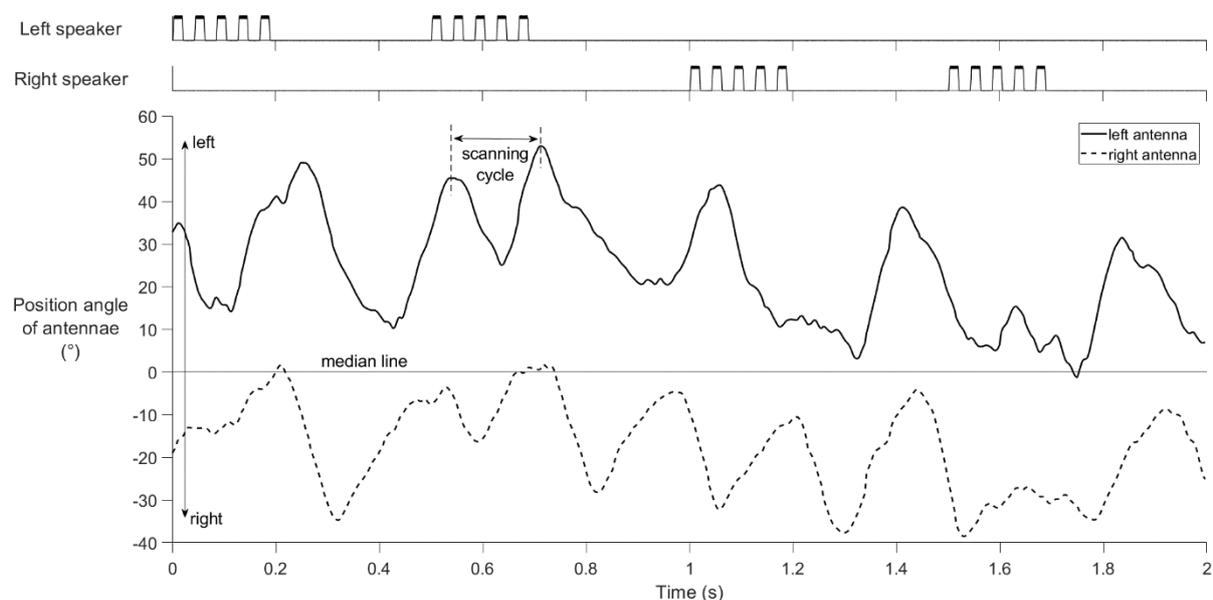


Fig. 2.5 Position angle of antennae during one cycle of the l-r2CP.

The mean duration of a scanning cycle of the antennae, as measured peak-to-peak in the position angle (Fig. 2.5), is 285-295 ms (Fig. 2.6). It did not differ significantly between the two antennae (two-way ANOVA, $F_{1,28} = 0.03$, $P = 0.855$) and was not affected significantly by the sound direction (two-way ANOVA, $F_{1,28} = 0.18$, $P = 0.671$). There was also no significant interaction between the two antennae and the sound direction (two-way ANOVA, $F_{1,28} \approx 0$, $P = 0.952$).

The mean angular range of the antennal scanning movements (difference between the maximum and minimum position angles) during the l-r2CP is 31-35° (Fig. 2.7 top). The angular range did not differ significantly between the two antennae (two-way ANOVA, $F_{1,28} = 1.89$, $P = 0.181$) and was not affected significantly by the sound direction (two-way ANOVA, $F_{1,28} = 0.57$, $P = 0.456$). The interaction between the left/right antennae and the left/right sound direction was also not significant (two-way ANOVA, $F_{1,28} \approx 0$, $P = 0.946$).

The antenna ipsilateral to the active speaker had on average a position angle 23-25° to the side of the speaker, whereas the contralateral antenna was closer to the median line, with a position angle 11-14° to the side contralateral to the active speaker (Fig. 2.7 bottom). The two antennae differed significantly in their position angles (two-way ANOVA, $F_{1,28} = 1296.5$, $P < 0.0001$), and the

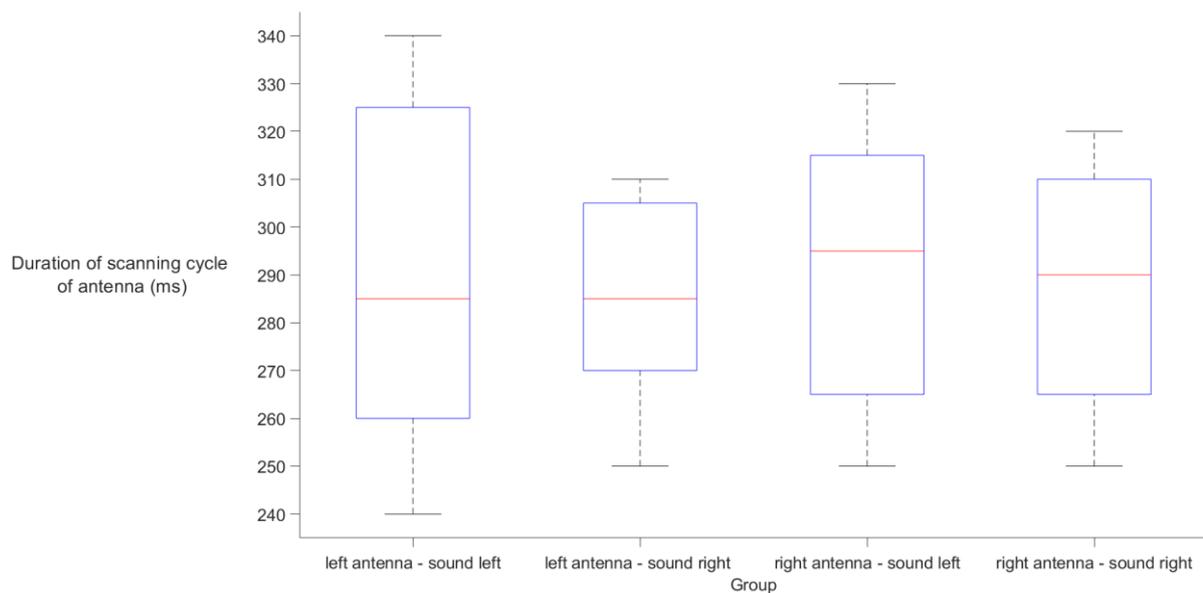


Fig. 2.6 Box plots for the durations of the antennae scanning cycles during the l-r2CP ($n = 8$ crickets, $N = 10$ scanning cycles per antenna per cricket). The four groups did not differ significantly from each other (see text for statistics)

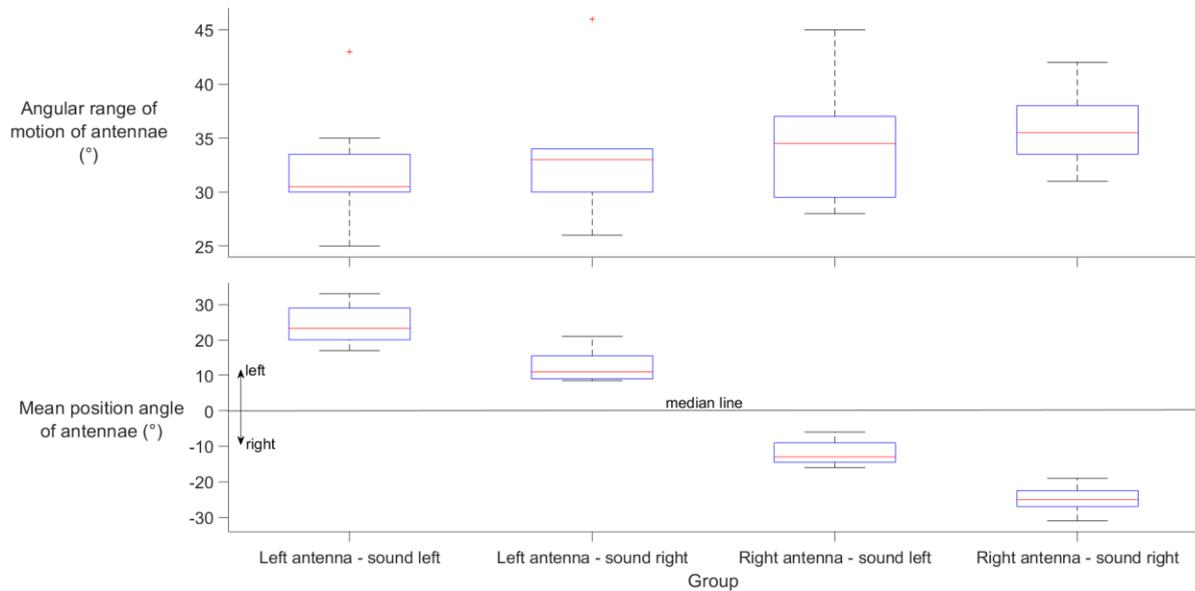


Fig. 2.7 Box plots for the angular range of motion and the mean position angle of antennae during the l-r2CP ($n = 8$ crickets, $N = 3$ repetitions of the paradigm per cricket). There is no significant difference in the angular range among the four groups, but there is a significant difference among the mean position angles (see text for statistics).

sound direction had a significant effect on the position angle (two-way ANOVA, $F_{1,28} = 14.98$, $P < 0.001$).

The interaction between the left/right antennae and the left/right sound direction also was significant (two-way ANOVA, $F_{1,28} = 23.81$, $P < 0.0001$).

Prothoracic movements during the l-r2CP

The prothorax followed the side of the active speaker and moved from a maximum position of around 5° to the left to a maximum position of 5° to the right during a cycle of the l-r2CP (Fig. 2.8 and Fig. 2.9 top). The maximum position angle (by absolute value) when the sound was presented from the left differed significantly from the maximum position angle when the sound was presented from the right (paired sample t -test, $P < 0.0001$, $n = 8$ crickets, $N = 3$ repetitions of the l-r2CP per cricket; Fig. 2.9 top). The mean position angle when the sound was presented from the left was 2° to the left and when the sound was presented from the right was 2° to the right; the two position angles differ significantly from each other (paired sample t -test, $P < 0.005$; Fig. 2.9 bottom).

Apart from steering to the direction of the calling song, the prothorax also makes smaller rhythmic movements that are coupled to the stepping cycle. To investigate the relationship between

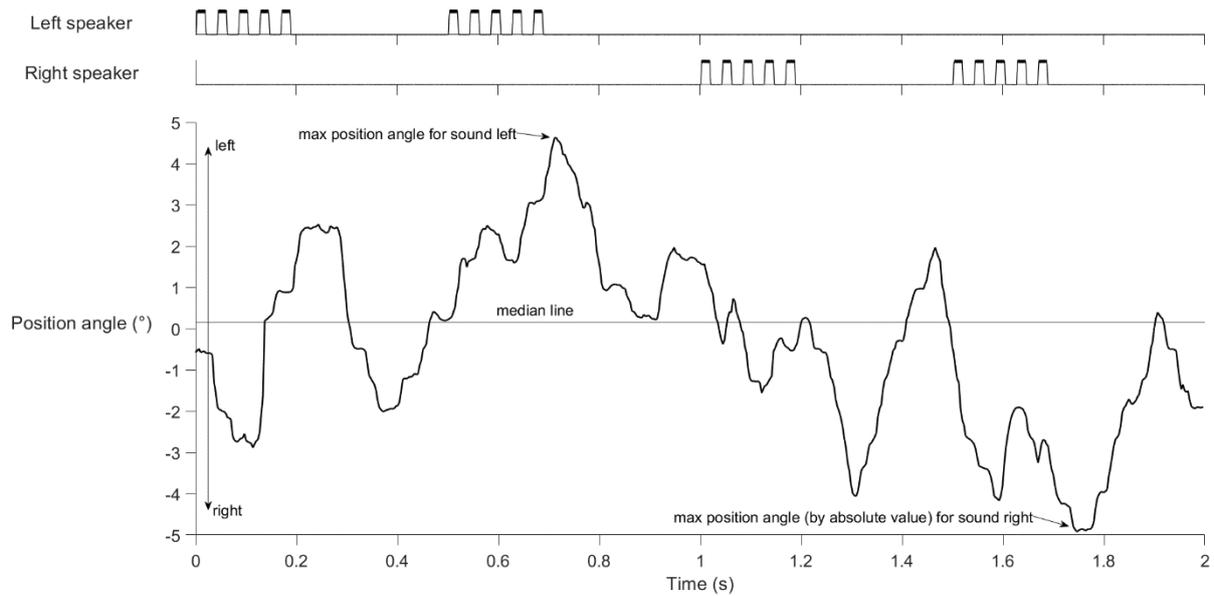


Fig. 2.8 Position angle of the prothorax during one cycle of the l-r2CP.

these movements and the stepping cycle, I used video sections where a cricket walked straight ahead, without steering, during the silent intervals of the SSP. This ensured that the tracked prothoracic movements were due to the stepping cycle alone.

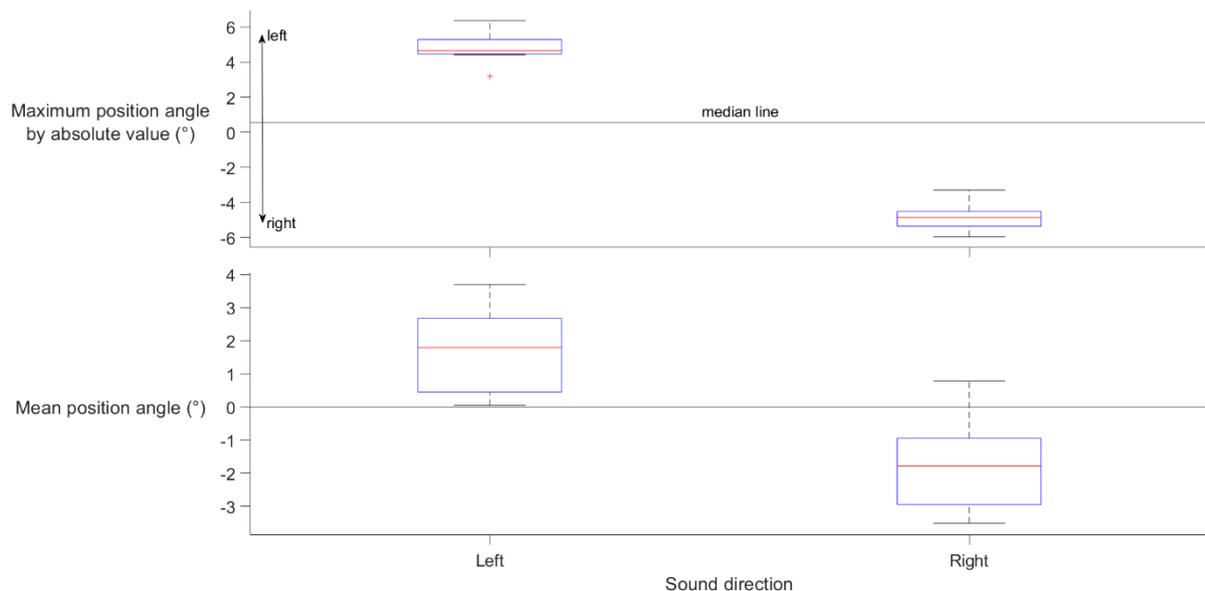


Fig. 2.9 Maximum position angle by absolute value (top) and mean position angle (bottom) when the sound is presented from the left and from the right during the l-r2CP ($n = 8$ crickets, $N = 3$ repetitions of the l-r2CP per cricket). The sound direction has a significant effect on both quantities (see text for statistics).

During the stepping cycle the prothorax generally tilts contralaterally to the front leg that is in the swing phase and ipsilaterally to the front leg that is in the stance phase (Fig. 2.10). The correlation coefficients between the angular position of the prothorax and the anteroposterior movements of the left and right front tarsi are -0.64 ± 0.11 and 0.58 ± 0.09 (mean \pm SD, $n = 8$ crickets, $N = 3$ sequences of 1 s straight ahead walking per cricket). During a step the average tilt of the prothorax to the left is $1.6 \pm 0.9^\circ$ and to the right is $-1.4 \pm 0.8^\circ$ (mean \pm SD, $n = 8$ crickets, $N = 10$ steps per cricket). The absolute values of the average tilts of the prothorax to the left and to the right do not differ significantly from each other (paired sample t -test, $P = 0.780$, $n = 8$ crickets, $N = 10$ steps per cricket).

Responses to the SSP

During the silent periods of the SSP the crickets stopped walking and remained motionless for intervals ranging 0.5-10 s. They consistently resumed phonotactic walking in response to the first chirp once the calling song started playing from the left speaker. I analysed which of the body parts or appendages was the first to move at the beginning of the phonotaxis. The first appendages to be activated at the start of phonotaxis and auditory steering were the antennae, approximately 180 ms after the start of the first chirp, with the head, prothorax, front legs, and middle legs following in that

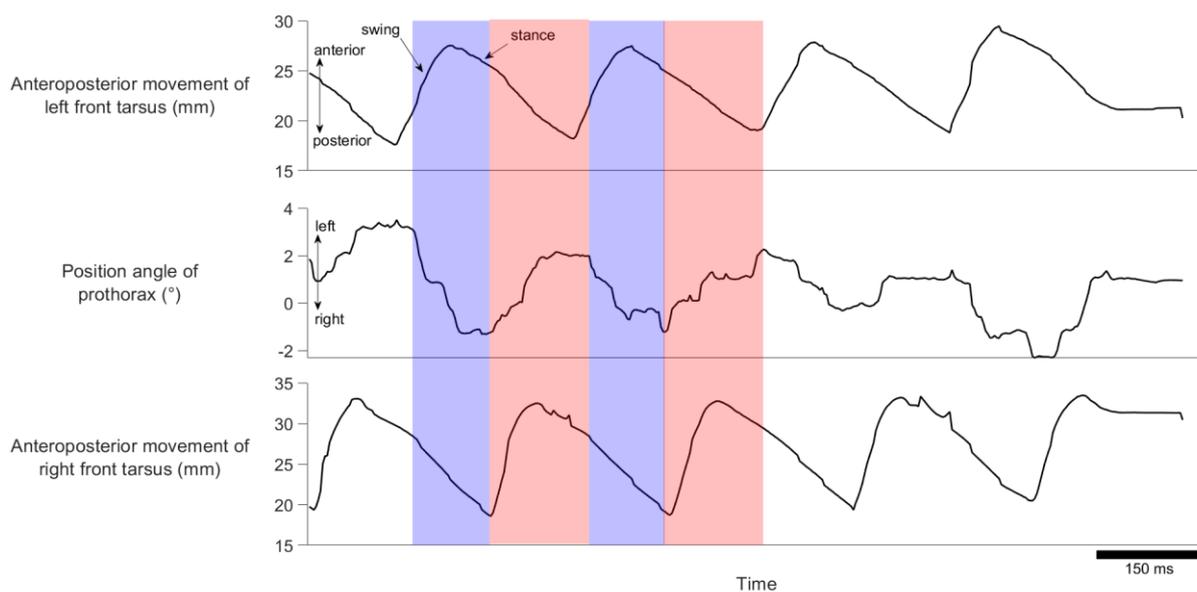


Fig. 2.10 Movement of the tarsi of the front legs and of the prothorax during straight ahead walking. Blue and red highlight the leftwards and rightwards movement of the prothorax respectively.

order (Fig. 2.11 and Table 2.2). Crickets also started walking spontaneously during the silent periods of the SSP, steering either to the left or to the right, and in these cases the sequence of movements could be analysed without sound stimulation. The order of activation of the body parts and appendages for spontaneous steering to the left was the same as with auditory steering to the left, although the activation delays relative to the activation of the left antenna were slightly longer for spontaneous walking (Table 2.2). The activation delays of the tracked body parts and appendages differ significantly from each other (two-way ANOVA, $F_{6,99} = 32.4$, $P < 0.001$), and so do the delays between auditory and spontaneous steering (two-way ANOVA, $F_{1,99} = 18.2$, $P < 0.01$).

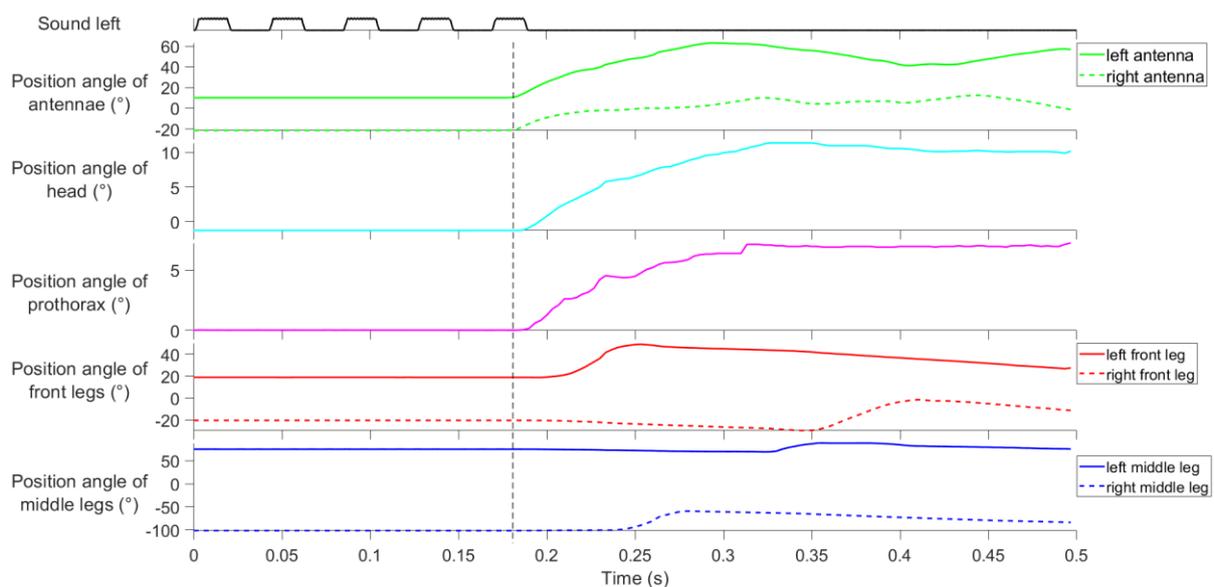


Fig. 2.11 Position angles of body parts and appendages in response to the first chirp from the left after 10 s of silence. The dashed vertical line indicates the point when the first appendage, the left antenna, is activated.

Table 2.2 Activation delays in response to the calling song and in spontaneous walking (mean \pm SD, $n = 8$ crickets, $N_1 = 3$ sequences of phonotaxis per cricket and $N_2 = 3$ sequences of spontaneous walking per cricket). The delays have been estimated with a temporal resolution of 3.33 ms, that is the temporal resolution of the 300 fps video recordings.

	Delay relative to start of first chirp from the left (ms)	Delay relative to activation of left antenna – phonotaxis (ms)	Delay relative to activation of left antenna – spontaneous walking (ms)
Left antenna	181 \pm 65	0	0
Right antenna	182 \pm 67	1 \pm 2	1 \pm 2
Head	186 \pm 62	5 \pm 5	12 \pm 6
Prothorax	188 \pm 60	7 \pm 7	15 \pm 6
Left front leg	191 \pm 62	10 \pm 7	22 \pm 8
Right front leg	201 \pm 52	20 \pm 11	26 \pm 7
Left middle leg	201 \pm 56	20 \pm 16	30 \pm 9
Right middle leg	208 \pm 57	27 \pm 22	30 \pm 11

Discussion

I have used high-speed video recordings to track the movement of the body parts and appendages of crickets during phonotaxis. The video analysis showed that the antennae and the prothorax exhibit auditory responses and move to the side of the active speaker. When initially motionless crickets commence phonotaxis, the antennae are the first appendages to move, with the head, prothorax, front legs, and middle legs following in that order.

Movement of the antennae

During phonotaxis crickets perform two types of antennal movements: a continuous side-to-side rhythmic movement that is consistent with previously reported scanning movements (Horseman, Gebhardt and Honegger, 1997), and an auditory-induced movement towards the sound direction,

which is superimposed on the rhythmic movement (Fig. 2.4A and 2.5). While the abduction can be performed over a wide range, the antennae cross the median line only slightly; their movement in the horizontal plane is restrained by the biomechanics of the pedicel-scape joint and the muscles inside the scape (Honegger *et al.*, 1990). The correlation coefficient between the movements of the two antennae showed that they are moderately positively correlated. A previous study reported that “the left and right antenna appear not to be strictly coupled” (Horseman, Gebhardt and Honegger, 1997), however this was based on observations and not quantitative analysis, and the data presented there suggest a moderate positive correlation as well. In walking stick insects, the coordination of the antennae is attributed to the bilateral coupling of functionally analogous joints (e.g the left head-scape joint and the right scape-pedicel joint), whereas the bilateral coupling of homologous joints (e.g. the left and right head-scape joints) is significantly weaker (Krause and Dürr, 2012). It is not known how the movement of the antennae is coordinated in the cricket; however, ablation experiments in stick insects showed that a combination of synaptic drive occurring in the brain – and not in the suboesophageal ganglion where the antennal nerves arise– and feedback from proprioceptors of the antennal joints are responsible for the control of the coordination of antennal movements (Krause, Winkler and Dürr, 2013).

The duration of the scanning movements of the antennae is shorter than that of the stepping cycle and does not depend on the acoustic stimulus (Fig. 2.6). The scanning movements possibly facilitate the navigation of *G. bimaculatus* through the grassland that is its natural habitat and where various obstacles are encountered (Hirtenlehner, Römer and Schmidt, 2014). During phonotaxis specifically, when the antennae touch an obstacle, the crickets cease walking to explore it (Haber Kern and Hedwig, 2016). The use of the antennae as tactile probes has been demonstrated in other insects as well, such as bees (Kevan and Lane, 1985; Erber *et al.*, 1997, 1998), cockroaches (Okada and Toh, 2006; Harley, English and Ritzmann, 2009), and stick insects (Krause and Dürr, 2012), and the tactile cues have been shown to be used for the adjustment of locomotion (beetles: Pelletier and McLeod, 1994; stick insects: Dürr, König and Kittmann, 2001; Schütz and Dürr, 2011).

The auditory-induced movement of the antennae towards the sound direction resembles their tracking of a moving visual target (Honegger, 1981); therefore, antennal movements are likely induced not only by auditory or tactile stimuli. The angular range of the antennal scanning movements is independent of the sound direction (Fig. 2.7 top), but the mean position angle of the antennae depends on the sound direction (Fig. 2.7 bottom). This indicates that the sound stimulus introduces to the antennal movements a bias towards the sound source, however the angular range of the antennal movements and the duration of the scanning cycles are unaffected. Both the scanning and the auditory-induced movements in the horizontal plane are controlled by the adductor and abductor muscles of the pedicel that are located inside the scape (Honegger *et al.*, 1990). The adductor muscle is innervated by one fast and two slow motoneurons, and the abductor muscle is innervated by one fast motoneuron and three motoneurons that exhibit properties of both slow- and fast-type motoneurons; all motoneurons have axons in nerves N2 or N4 arising at the deutocerebrum and somata in the deutocerebrum (Honegger *et al.*, 1990). The slow motoneurons can induce prolonged changes to the antennal position and sustained contraction of the muscles they innervate (Honegger *et al.*, 1990), they could therefore be responsible for the auditory-induced DC shift in the antennal position. On the other hand, the fast motoneurons could provide twitch contractions that induce the scanning movements. However, this is a hypothesis and only intracellular recordings of the motoneurons – ideally in behaving animals – would confirm this.

Movement of the body

The lateral movements of the head and the prothorax are moderately positively correlated (Fig. 2.4B,C right), meaning they are generally moving towards the same direction in the horizontal plane. The prothorax exhibits two types of lateral movement: one that is oscillatory and is coupled to the stepping cycle, with an average amplitude of around 1.5° (Fig. 2.10), and one that is auditory-induced and is superimposed onto the first, and during which the prothorax can reach a position angle of around 5° (Fig. 2.8 and 2.9 top).

During a step, the prothorax is bending away from the front leg that is in the swing phase and towards the front leg that is in the stance phase (Fig. 2.10). This prothoracic movement allows the swinging tarsus to reach further forward as compared to if the prothorax was rigid during the step. Therefore this prothoracic movement may be an adaptation that increases the efficiency of each step, i.e. more distance is covered with each step. The prothoracic oscillation during walking is not a passive movement, and as shown in the next chapter, it is at least controlled by pronotal muscle 56 (Furukawa, Tomioka and Yamaguchi, 1983). Even though the central pattern generators, the peripheral feedback, and the descending signals that control the coordination of the legs during walking have been studied for decades (review: Bidaye, Bockemühl and Büschges, 2018), the contribution of the prothorax to the stepping cycle or to steering has generally not been explored. It has been reported however that the praying mantis *Tenodera aridifolia* bends the prothorax to the direction of a prey before initiating walking towards it (Yamawaki *et al.*, 2011).

Movement of the tarsi of the front and middle legs

The anteroposterior movements of the tarsi of the front and middle legs are coupled to the stepping cycle and reflect the swing and stance phase of each leg (Fig. 2.4 D, E left). In general, during walking, one front leg is in the swing phase, while the other one is in the stance phase, and each middle leg is in phase with the contralateral front leg. Sound has a significant effect on the lateral position of the tarsi of both front legs, as they move towards the active speaker. A similar movement was observed for the tarsi of the middle legs, however it was not statistically significant. Overall, the movement of the tarsi in this study is in line with what previous studies have reported (Witney and Hedwig, 2011; Petrou and Webb, 2012).

Order of activation of tracked body parts and appendages

Motionless crickets initiate phonotaxis by first moving the antennae towards the side of the active speaker, and the rest of the body parts and appendages are activated from anterior to posterior in the following order: head, prothorax, front legs, and middle legs (Fig. 2.11 and Table 2.2). From a teleological perspective, the early activation of the antennae allows the crickets to scan and explore

their surroundings before they initiate locomotion. During phonotaxis if the antennae of a cricket touch an object, they cease walking to explore the object (Haber Kern and Hedwig, 2016). Among other insects, antennal tactile cues are used by beetles to adjust their body axis (Pelletier and McLeod, 1994) and by stick insects to adjust their leg position (Dürr, König and Kittmann, 2001; Schütz and Dürr, 2011). Moreover, the antennae move with little mass against minimal resistance, and as a result antennal movement is likely to be more rapidly generated from a neuro-muscular motor signal.

The neural circuitry responsible for the coordination of the segments and appendages during walking likely resides in the thoracic nerve cord, however, inputs descending from the brain are instrumental in the activation and manipulation of this circuitry to achieve goal directed locomotion (review: Bidaye, Bockemühl and Büschges, 2018), which in this case is the steering towards the sound source. Böhm and Schildberger (1992) identified a descending neuron whose activity is correlated with the commencement of walking, and, perhaps more relevant, Zorović and Hedwig (2011) identified three descending interneurons that exhibit auditory responses and initiate walking and steering when injected with current. However, the order of activation of the tracked body parts and appendages is not specific to auditory-induced steering, since it is the same as when the crickets are engaging in spontaneous walking, albeit with slightly longer delays relative to the activation of the antennae (Table 2.2). In the case of the auditory-induced steering, however, the descending command is gated by the pattern recognition circuit in the brain (Schöneich, Kostarakos and Hedwig, 2015), and the directional cues regarding the location of the sound source would have to be integrated into the motor response as well.

On average, the antennae start to move approximately 180 ms after the start of the first chirp in a sequence. In a previous study where the walking paths of the crickets were tracked with a trackball, their response delay during ongoing phonotaxis to the sound pulses of the calling song was just 55-60 ms (Hedwig and Poulet, 2004). Since the crickets rotate the trackball with their legs, the corresponding delay of this study is that of the front legs, which is approximately 190 – 200 ms (Table 2.2). However, in the previous study the delay was measured in crickets that were constantly

presented with the calling song during phonotaxis, whereas in this study the delay was measured in crickets that were presented with the calling song after they became motionless during an interval of silence. Therefore, the motionless crickets had to overcome their inertia, which potentially resulted in slower reaction times. In addition, pattern recognition may have to be established again in the motionless crickets, and the decision to approach the sound source has to be made, leading to longer response delays. Furthermore, pattern recognition modulates phonotactic steering at least transiently (Poulet and Hedwig, 2005), therefore once pattern recognition has been established (i.e. in crickets that are constantly presented with the calling song), the auditory signal underlying rapid steering responses may be processed at the thoracic level and may not have to ascend to the pattern recognition circuit in the brain (Schöneich, Kostarakos and Hedwig, 2015), resulting in faster reaction times.

Chapter 3: Activity of antennal and thoracic muscles during auditory steering

Abstract

The activity patterns of the antennal muscles of the scape and of several thoracic muscles were investigated for contribution to auditory-induced movements of the antennae and the prothorax, respectively. The movements of the antennae of crickets performing phonotaxis were tracked, and at the same time the activities of the antennal adductor and abductor muscles of the scape were recorded. The antennae moved in the direction of the active speaker; an antenna was adducted towards the median line when the calling song was presented contralaterally to the antenna and abducted away from the body when the calling song was presented ipsilaterally. The antennae exhibited auditory-induced movement towards individual chirps of the calling song both when it was presented constantly from one speaker and during acoustic paradigms where the presentation alternated rapidly between a speaker to the left and a speaker to the right of the cricket. The activity of the adductor muscle was coupled to the adduction movement during the contralateral presentation of the song, while the activity of the abductor muscle was coupled to the abduction of the antenna during the ipsilateral presentation. Auditory responses to individual chirps suggest that during phonotaxis the antennal motoneurons receive constant input related to the side of the sound. However, there is no overlap between the projections of the motoneurons and the ascending auditory pathway in the brain, meaning brain interneurons must forward the auditory motor signal to the motoneurons. The delay of the antennal movement relative to the calling song ranged from 43 ms when the calling song was presented continuously from one side to 183 ms when the presentation of the calling song switched side, and the delay of the muscular activity ranged from 90 to 180 ms, respectively. The antennal movement could precede the activation of the muscles; such movement was likely initiated by passive forces exerted by the muscles and the scape-pedicel joint.

Of all the thoracic muscles tested, only pronotal muscle 56 exhibited auditory-induced responses in crickets performing phonotaxis on the trackball. Its activity was coupled to the movement

of the prothorax both when it made rhythmic side-to-side movements in accordance with the stepping cycle and during auditory-induced steering. The spike count of muscle 56 increased markedly after a delay of 37-75 ms relative to the presentation of the calling song and the ensuing auditory-induced prothoracic steering had a delay of 50-84 ms. Auditory input to the motoneurons of muscle 56 is likely indirect, since the projections of the motoneurons in the prothorax do not overlap with the auditory neuropil, and, based on the delay times, likely via an unidentified descending pathway from the brain. Among the other thoracic muscles tested, the prothoracic intersegmental muscle 59 and the prosternal muscle 87b possibly contribute to the steering of the prothorax, however they could not be tested in walking crickets.

Introduction

The tympanal ears on the tibia of the front legs of crickets serve two functions: predator detection and intraspecific communication. Predator avoidance refers to their ability to listen to the ultrasonic echolocation cries of bats and respond by steering away from them (Nolen and Hoy, 1984). On the other hand, hearing in the context of intraspecific communication is utilized to receive the rivalry song in male-male interactions, and the calling and courtship songs in male-female interactions (Alexander, 1962). The females recognize the conspecific calling song by temporal features, such as the pulse duration and period, and perform phonotaxis towards the singing males (Pollack and Hoy, 1979). The acoustic behavior of crickets has made them attractive organisms for the study of, among others, the biophysics and neural control of sound production [biophysics: Montealegre-Z, Jonsson and Robert, 2011; neural control: Kutsch and Huber, 1990; Hedwig, 2000], the motor pattern generator of acoustic signals (Schöneich and Hedwig, 2011, 2012), the neural control of bat avoidance behavior (Nolen and Hoy, 1984), directional hearing (Michelsen, 1994; Michelsen and Löhe, 1995; Schöneich and Hedwig, 2010), frequency processing (Nocke, 1972; Pollack and Faulkes, 1998), and pattern recognition (Schöneich, Kostarakos and Hedwig, 2015). However, despite the progress in the field, little is known about the integration of the auditory information into the motor responses of the females during phonotaxis.

The cricket ear has 45-60 receptor neurons, which project ipsilaterally to the prothoracic ganglion, with the majority of them tuned to frequencies similar to that of the calling song, and a subset tuned to ultrasound for bat detection (Imaizumi and Pollack, 1999). Ascending Neuron 1 (AN1) transfers auditory signals of the conspecific frequency from the prothorax to the brain (Schildberger and Hörner, 1988), where the recognition of the conspecific pattern takes place (Schöneich, Kostarakos and Hedwig, 2015). In *Gryllus bimaculatus*, the species of this study, the calling song consists of pulses grouped into chirps, and a group of neurons that form a ringlike arborization in the anterior protocerebrum implement a coincidence detection mechanism for the detection of the conspecific pulse rate (Schöneich, Kostarakos and Hedwig, 2015).

From the brain, it is unclear how the command for the motor control of phonotaxis descends once auditory pattern recognition has been established. Overall, around 200 interneurons descend from the brain (Staudacher, 1998), some of which have been shown to exhibit responses to auditory stimuli (Staudacher and Schildberger, 1998; Staudacher, 2001; Zorović and Hedwig, 2011). However, the descending auditory responses are generally weak and it is not clear if they are sufficient to drive the fast auditory steering that lags 55-60 ms from the presentation of the first pulse of a chirp (Hedwig and Poulet, 2005).

Phonotaxis requires two types of motor activity: locomotion and auditory-induced steering. Auditory steering is coupled to individual pulses of the calling song (Hedwig and Poulet, 2005), unlike the movement of the front legs (Baden and Hedwig, 2008), therefore the control of auditory steering may be decoupled from the central pattern generator for locomotion. High-speed video recordings of crickets performing phonotaxis showed that the antennae and the prothorax exhibit auditory responses and move to the direction of the calling song (see Chapter 2). Based on these findings, here I investigate the function of antennal and prothoracic muscles that potentially contribute to such auditory responses. Specifically, I recorded the activities of the antennal adductor and abductor muscles [#6 and #7 in Honegger et al. (1990)] in crickets performing phonotaxis, and at the same time

tracked the antennal movements with an optoelectronic device (Hedwig, 2000). I also investigated the function of several muscles of the prothorax and mesothorax via recording their activity, stimulating them with current, manually pulling them with forceps, or analyzing their insertion points with Computed Tomography (CT) and by visual inspection. The aim was to identify muscles that control the lateral bending of the prothorax against the mesothorax. I focused the study on pronotal muscle 56 (Furukawa, Tomioka and Yamaguchi, 1983), based on the results of preliminary tests and its accessibility for recording during phonotaxis. The position and course of the muscles examined in this study were visualized with 3D models constructed from CT scans.

Materials and methods

Crickets and tracking of body parts and appendages

The rearing and preparation of the crickets for testing on the trackball are described in Chapter 2, however in this case no white spots were painted on the body parts and appendages of the crickets. Instead, a custom-built optoelectronic system (Hedwig, 2000) was used to track either the movement of the pronotum, antenna, head, or front leg femur depending on which muscle was being tested. A modified SLR camera with a photodiode (Laser Components, Olching, Germany; Type 1L30) in the plane of the film was used to record the movements of a circular sticker (\varnothing 1 mm) made from a reflective material (Scotchlite 7610; 3M Laboratories, Neuss, Germany) glued to the appendage/ body part being tracked. To track the antennal movements, I used wax to fix the prothorax to the mesothorax, the head to the prothorax, and the scape to the head. This ensured that the optoelectronic system tracked only the lateral movement of the antenna. Similarly, when the head movement was tracked, the prothorax was fixed to the mesothorax with a drop of wax. The optoelectronic device and the light source, which had a red filter because red light is not visible to *G. bimaculatus* (Zufall, Schmitt and Menzel, 1989), were positioned posteriorly of the animals and the movement of the appendage of interest was tracked via the reflection of the sticker on a mirror positioned at an angle of 45° above the cricket.

Trackball system

The trackball system uses a Rohacell ball such as the one described in Chapter 2, with the add-on of an optical sensor (Agilent ADNS-2051, 2-D Optical Mouse Sensor) that tracks the rotation of the ball. Whenever the ball moved by 116 μm forward-backward or left-right, a 150 μs long TTL pulse was generated in the X- or Y-channel respectively (Fig. 3.1). A pulse of positive amplitude encoded forward movement in the X-channel and left movement in the Y-channel, whereas a negative pulse encoded backward movement in the X-channel and right movement in the Y-channel. The walking and steering speed of the cricket was calculated from the coding pulses.

Investigation of muscle function

The function of 11 muscles was tested (highlighted in Fig. 3.2), with the aim to identify muscles that contribute to the lateral movements and auditory responses of the antennae and the prothorax. The method of testing the function of a muscle depended on its location and accessibility. Ideally, when

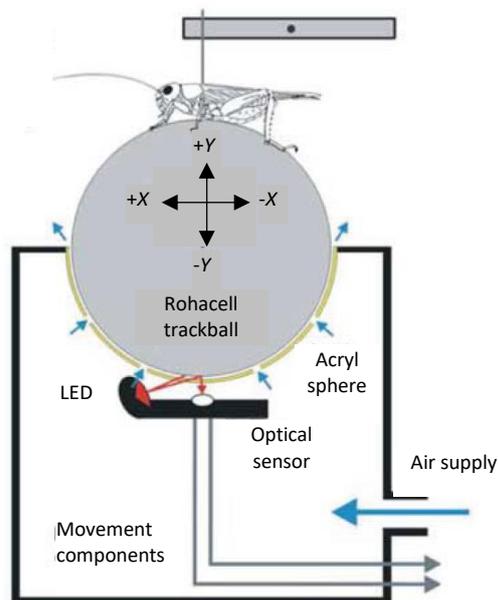


Fig. 3.1 The trackball system. A cricket is tethered on top the Rohacell ball. Whenever the cricket walks, the optical sensor picks up the left/right and forward/backward movements of the ball and encodes them into pulses. Adapted from Hedwig and Poulet (2004).

the location of a muscle allowed it, its activity was recorded while a cricket was performing phonotaxis. If testing with a walking preparation was not feasible, the cricket was tethered on a piece of plasticine, and following vivisection, the muscle of interest was either recorded (amplifier model 1700 and 1800, A-M Systems, Sequim, WA, USA), or stimulated with current (stimulus isolator model A 360, World Precision Instruments, Sarasota, FL, USA), or manually pulled with forceps, and its contractions and effects thereof were observed (Table 3.1). To record the activity of a muscle or to stimulate it with current, I inserted into the muscle two varnished steel wires with a diameter of 30 μm (Rheinische

Table 3.1 List of antennal and thoracic muscles and methods of testing their function

Muscle	Method of testing
6	EMG in walking crickets
7	EMG in walking crickets
55a,b	Stimulation with current (muscles 55a and 55b were tested as a single unit)
56	EMG in walking crickets
59	Observation in tethered crickets
60a	EMG in a tethered cricket
60b	EMG in a tethered cricket
71b1	EMG in a walking cricket
87a	Manually pulling muscle with forceps
87b	Stimulation with current

Feindraht Industrie, Reichshof, Germany) that were soldered to a male pin header (model AmpModu Mod II, TE Connectivity, Schaffhausen, Switzerland). The pin header was then connected to the amplifier or the stimulus isolator to record or stimulate the muscle, respectively. In walking crickets,

Fig. 3.2 (previous page) Antennal and thoracic musculature of a cricket. The muscles tested in this study are highlighted in red. **(A)** Dorsal view of the antennal base of a cricket, with scape (sc), pedicel (p), and the compound eye (ce) as landmark. The dorsal surface of the scape is removed to reveal the adductor (6) and abductor (7) of the pedicel (p). **(B)** Inner musculature in the right half of the neck and thorax. Median section, viewed from the inside. **(C)** Outer musculature in the right half of the neck and thorax following the removal of muscles shown in B. **(D)** Dorsal view of the ventral musculature of the thorax. The anterior parts of the muscles, 60a, b and 61 are omitted to show other muscles. a: anterior; Ba: basalare; ce: compound eye; CS: cervical sclerite; Cx1, Cx2, Cx3: prothoracic, mesothoracic, and metathoracic coxae; d:dorsal; EP: episternum; H: head; jm: joint membrane; l: lateral; m:medial; Msn: mesonotum; Mtn: metanotum; NM: neck membrane; p: posterior; pe: pedicel; Ph1 and Ph2: first and second phragmata; PlA1, PlA2, and PlA3: first, second and third pleural arms; PlR2 and PlR3: second and third pleural ridges; PoR: postoccipital ridge; Sa: subalare; SA1, SA2, and SA3: first, second, and third sternal apophyses; sc: scape; Spn1 and Spn2: external pits of first and second spinae; St1 and St2: first and second stigmata; v: ventral. **A** modified from Honegger *et al.* (1990), **B**, **C**, and **D** modified from Furukawa, Tomioka and Yamaguchi (1983).

the wires were inserted into the muscle through the cuticle, whereas in tethered animals the wires were inserted after opening the cuticle to reveal the muscle. In this study I have focused on antennal muscles 6 and 7 and pronotal muscle 56 because preliminary tests showed that they control the lateral movement of the antenna and the prothorax respectively, and their activity could be recorded in crickets performing phonotaxis.

Histology, CT scans, and 3D modelling of muscles

To stain the thoracic muscles for visualisation with a CT scan, the whole body of a cricket was submerged in 1% Lugol's iodine solution (Pro-Lab Diagnostics, Birkenhead, UK) in Ethanol and gently shaken (model 802 suspension mixer, Luckham) for a week, after which the stained sample was washed 3 X 10 min and drained-off in ethanol.

To prepare the antennal muscles for staining, the head of a cricket was fixed in 4% paraformaldehyde (Sigma-Aldrich, St Louis, USA) in 1 X phosphate-buffered saline (PBS; Life Technologies Limited, Paisley, UK) and gently shaken overnight. After fixation, the head was washed 3 x 10 min in 1 X PBS and dehydrated stepwise in methanol (25%, 50%, 75% and 100% methanol each for ~10 minutes). Then the head was stained with 1% (w/v) phosphotungstic acid (Sigma-Aldrich) in methanol by gently shaking it in the staining solution for a week and refreshing the solution twice during that period. Once the staining was complete, the head was washed and drained-off in methanol.

To scan the stained samples, they were taken out of their solutions and placed in an Eppendorf tube padded with cotton wool to ensure they would remain in position during the scanning process. The Eppendorf tube was fixed on the scanner turntable with plasticine. The stained samples were scanned (XTEK H 225 ST MicroCT scanner, Nikon, Minato, Tokyo, Japan) into 2000 images from anterior to posterior. The resolutions of the scans of the whole cricket and the head were 19 and 5 μm anterior to posterior respectively (X-ray voltage 120 kV, current 120 μA). The preparation of the samples and the scans were conducted at room temperature of 21 °C.

The original scan data were used to construct dorsal-to-ventral and left-to-right image stacks (ImageJ, public domain). This facilitated the identification of the muscles that were subsequently modelled in 3D (Mimics 23, Materialise, Southampton, UK).

Acoustic Stimulus

To elicit phonotaxis in walking crickets, I used the artificial calling song and two-speaker set-up described in Chapter 2. Before the main acoustic test was presented to a cricket, its responsiveness to the calling song and left/right symmetry of its response was assessed with a preliminary test by playing the song from the left and right speaker for 1 min each. If the cricket performed phonotaxis, but its lateral deviation towards the left and right speaker differed more than 20% (Fig. 3.3A), it was re-aligned on the trackball and tested again to ensure symmetry of its response (Fig. 3.3B). If the cricket was unresponsive, it was not further tested (Fig. 3.3C). An asymmetry in a cricket's response can possibly arise from a human error when aligning the cricket on the trackball and/or an asymmetry in its auditory and/or motor system.

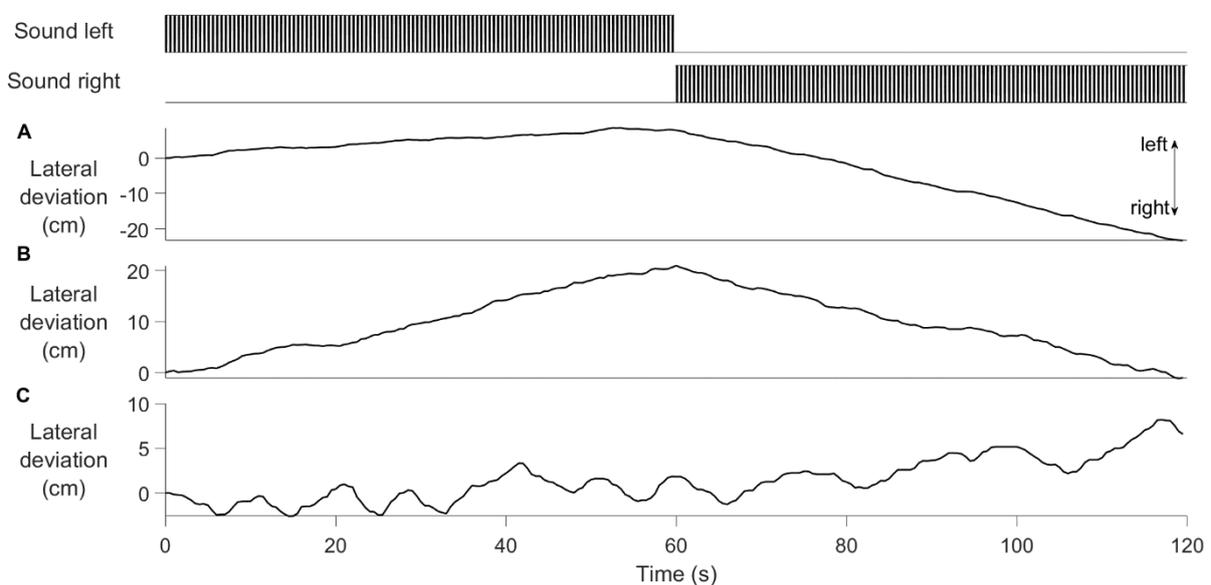


Fig. 3.3 Test for the assessment of the phonotactic responsiveness and the symmetry of auditory steering of female crickets. **(A)** A cricket orienting towards the calling song, but with a bias towards steering to the right. **(B)** Same cricket as in **(A)**; after re-aligning its position on the trackball, the asymmetry of its response is corrected. **(C)** Example of a cricket that did not perform phonotaxis. This cricket was not tested further.

To test the responses of pronotal muscle 56 and the antennal muscles 6 and 7 during phonotaxis, the crickets that “passed” the preliminary test were presented with the following acoustic stimuli, which aimed to elicit a high number of left/right auditory steering movements. Two paradigms, each lasting 1 min were used and presented in succession: i) 2 chirps left - 2 chirps right (abbreviated l-r2CP) repeated 30 times and ii) 1 chirp left – 1 chirp right (abbreviated l-r1CP) repeated 60 times. After a test, stimulation stopped for 1 min to allow the cricket to rest, and then the test was repeated. Each cricket was presented four times with the above acoustic sequence. Only the segments during which a cricket was responsive to the acoustic stimulus were used in the data analysis.

Data acquisition and analysis

In trackball tests where the function of the antennal muscles was tested, six data channels were acquired in each test: the acoustic stimuli played from the two speakers, the two trackball channels encoding the forward and lateral walking of the cricket, the EMG with the summed activity of adductor muscle 6 and abductor muscle 7, and the data from the optoelectronic device tracking the lateral movement of the right antenna. Similarly, in trackball tests where the activity of pronotal muscle 56 was recorded and the lateral movement of the pronotum was tracked, seven data channels were acquired, because the activities of the left and right muscle 56 were recorded in separate channels (Fig. 3.4). All signals were sampled at 10 kHz with a data acquisition system (Micro1401-3, CED, Cambridge, UK) controlled with the software Spike2 (CED).

The data analysis was performed off-line with Spike2 and Neurolab (Knepper and Hedwig, 1997). The forward and lateral steering velocities of the crickets were calculated by counting the pulses over time in the X- and Y-channel and multiplying them by the trackball constant, 116 μm . The forward walking and the lateral deviation of the crickets were then calculated by integrating the forward and steering velocity respectively. The lateral deviation was used to assess a cricket’s phonotactic responsiveness during the preliminary test.

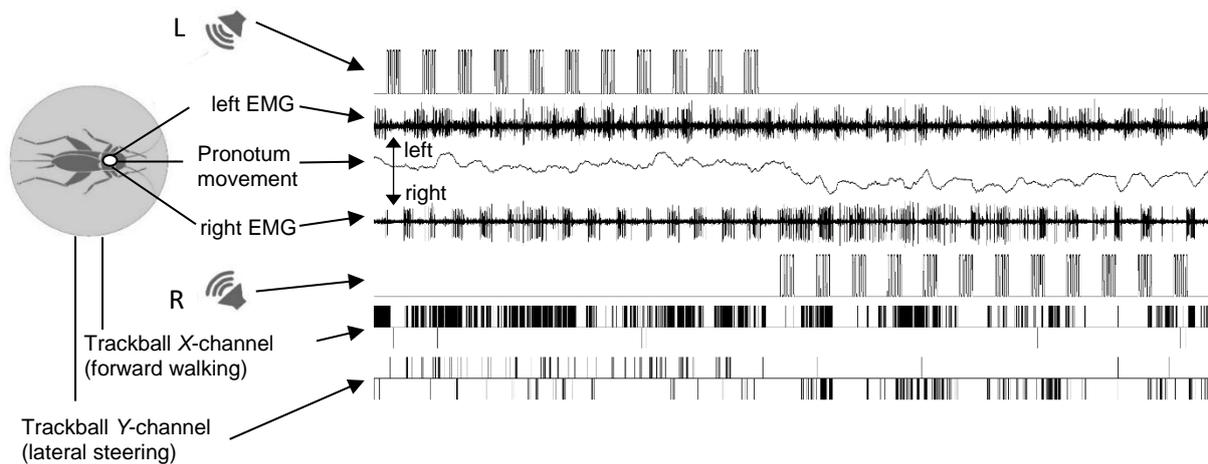


Fig. 3.4 Experimental set-up for investigating the contribution of pronotal muscle 56 to phonotaxis. Two speakers set at 45° play the calling song to a cricket on a trackball floating above a sensor that encodes into pulses the forward and lateral components of the cricket's walking. The activities of the left and right muscle 56 are recorded with varnished steel wires and the lateral movement of a reflective sticker on the pronotum is tracked with an optoelectronic device.

Results

Assessment of phonotactic motivation

Overall, 42 out of 104 crickets (41%) exhibited phonotaxis to the preliminary test (Fig. 3.3). In 12 out of the 42 responsive individuals, the EMGs were of sufficient quality to be included in the data analysis.

Antennal muscles of the scape

Each antenna consists of three basic segments: the scape (base), the pedicel (stem), and the long slender flagellum (Fig. 3.5). The scape forms a hinge joint with the head capsule that allows the former to move in the vertical plane (up and down). Accordingly, the pedicel forms a hinge joint with the scape that allows the pedicel and flagellum to move in the horizontal plane (left and right). Antennal adductor muscle 6 arises medially at the base of the scape and attaches medially at the base of the pedicel. In the 3D reconstruction of the muscle from CT scans, it appears to consist of 2 units (see Fig. 3.5 right), although it had previously been described as a single unit (Honegger *et al.*, 1990). When muscle 6 contracts, it pulls the pedicel and the flagellum towards the median line (adduction). Antennal abductor muscle 7 arises distally at the base of the scape and attaches distally at the base of the pedicel. Its contraction pulls the pedicel and the flagellum away from the median line (abduction).

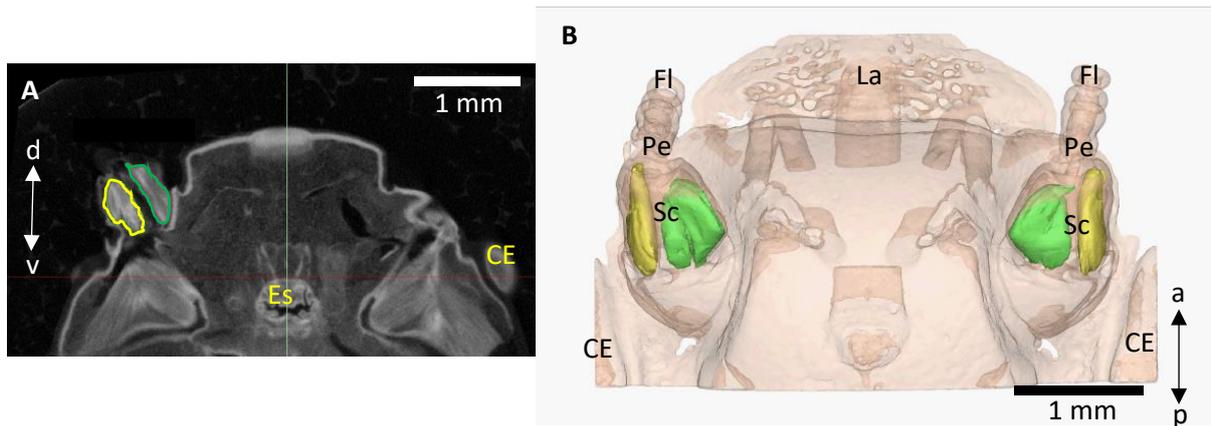


Fig. 3.5 Anatomy of antennal muscles of the scape. **(A)** Slice from CT scan of the head. Antennal adductor muscle 6 and abductor muscle 7 are highlighted in green and yellow respectively. **(B)** Top view of 3D model of the head with muscles 6 and 7 shown in green and yellow. CE: compound eye; Es: esophagus; Fl: flagellum; La: labrum; Pe: pedicel; Sc: scape; a: anterior; d: dorsal; p: posterior; v: ventral.

One of the aims of the tests was to relate the activity of the antennal muscles to the antennal movement and the sound stimulus. Obtaining a stable EMG was challenging, because the EMG signal would regularly be “lost”, possibly due to the recording wires moving out of the small muscles during the antennal movements. As a result, out of the 40 crickets upon which an EMG was attempted, only in 2 crickets the recording was of adequate quality to be analysed. In these 2 crickets, even though the EMG wires were inserted into muscle 7, they recorded the activity of both muscle 6 and muscle 7, possibly due to the close proximity of the two muscles. However, as an advantage, the responses of both muscles could be analysed.

When a cricket performs phonotaxis, it moves its antennae constantly side-to-side (Fig. 3.6). During the preliminary tests, where the calling song was presented continuously for 60 s from the left and then from the right, the lateral movement of the tracked right antenna increased in amplitude when the calling song was presented ipsilaterally. The activity of muscle 6 is coupled to the adduction of the right antenna (i.e. movement towards the median line), whereas the activity of muscle 7 is coupled to the abduction (i.e. movement away from the median line).

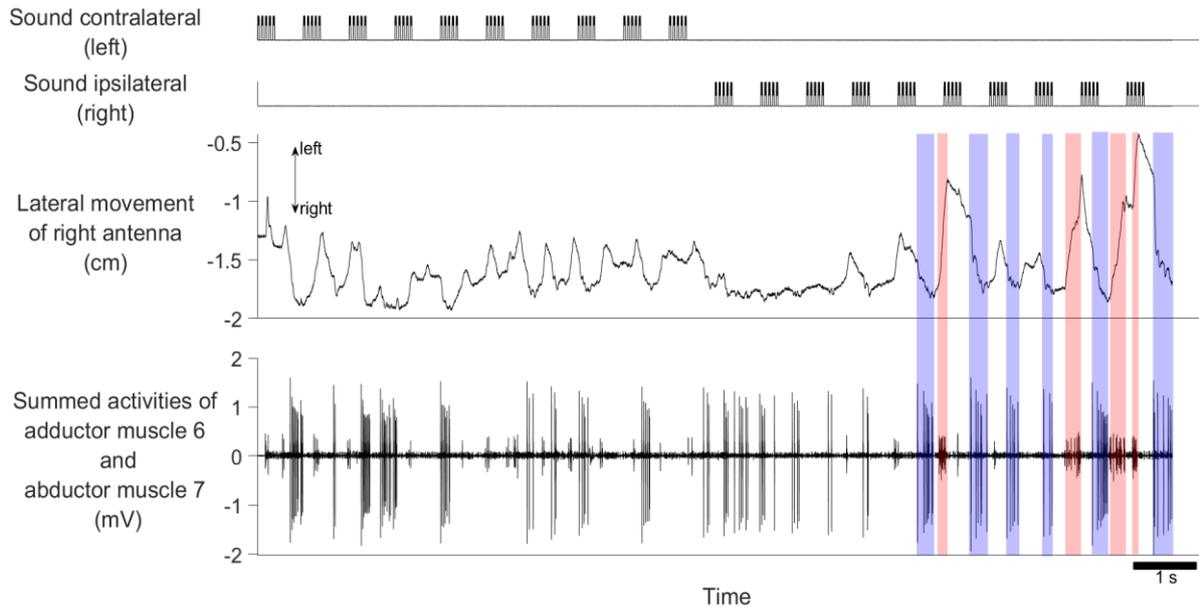


Fig. 3.6 Antennal movement and activity of antennal muscles 6 and 7 in a cricket performing phonotaxis. The antennal movement has been measured as the distance from the median line of the animal. The small units in the EMG belong to antennal adductor muscle 6, whereas the large units belong to abductor muscle 7. Activity highlighted in red shows examples of muscle 6 firing during adduction of the right antenna, and activity highlighted in blue shows muscle 7 firing during abduction of the antenna.

On average, following the presentation of a chirp from the left, the right antenna was adducted slightly to the left (Fig. 3.7 left column). In cricket 1 the adduction movement started 53 ms after the start of the chirp, and the antenna reached a maximum deviation of 1.5 ± 0.4 mm (mean \pm SEM; $N = 120$ chirps from the left) relative to its position at the start of the chirp. In cricket 2 adduction started 116 ms after the start of the chirp and the antenna reached a maximum deviation of 1.0 ± 0.7 mm. The activity of muscle 6, which controls the adduction of the antenna, increased markedly in cricket 1 90 ms after the start of a left chirp. In cricket 2, however, there is no marked increase in the activity of muscle 6 following the presentation of a left chirp. This could mean that, when the calling song is played from the contralateral side, the function of muscle 6 is to constantly keep the antenna adducted towards the median line rather than fire in accordance to the song pattern on a chirp-to-chirp basis. Still, muscle 6 is more active in both crickets when the calling song is played contralaterally than ipsilaterally, with 3.7 versus 2.5 spikes/chirp for cricket 1 and 4.9 versus 3.3 spikes/chirp for cricket 2.

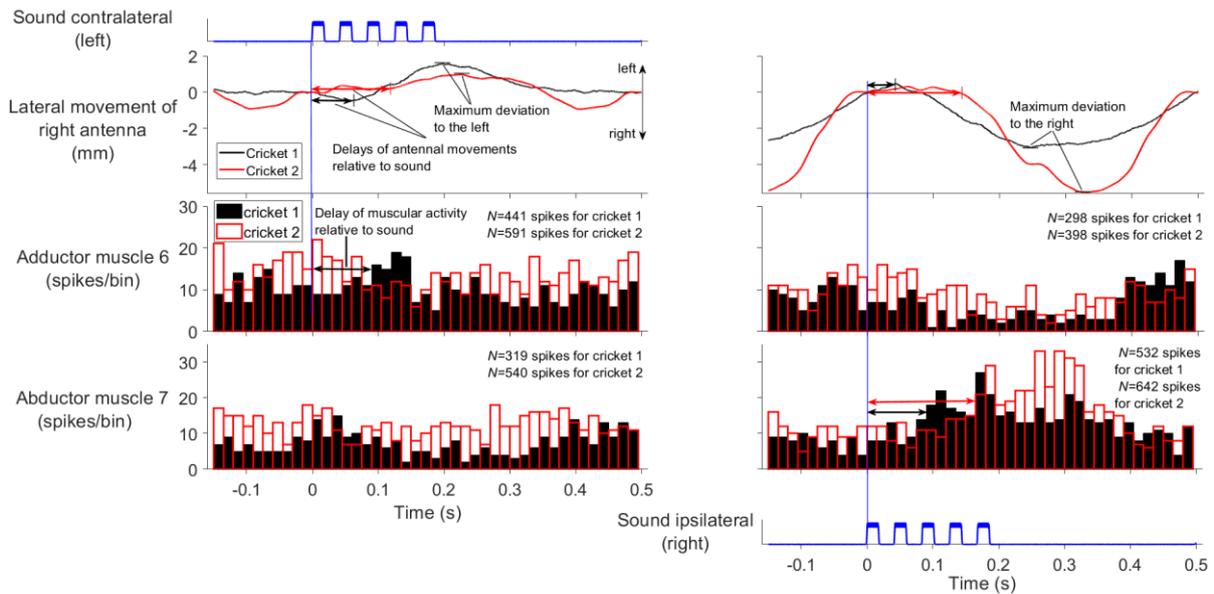


Fig. 3.7 Average antennal movements and PST histograms for continuous stimulation with the calling song. Left column: calling song presented contralaterally to the right antenna ($N = 120$ chirps). Right column: Calling song presented ipsilaterally to the right antenna ($N = 120$ chirps). The lateral movement of the antenna has been measured as the deviation from its position at the beginning of the chirp. The bin size for the PST histograms is 15 ms.

The movement of the right antenna was more pronounced for both crickets when the calling song was presented ipsilaterally, i.e. from the right (Fig. 3.7 right column). Following the presentation of a chirp from the right, the antenna started to move to the right (abduction) and reached a maximum deviation of 3.1 ± 0.6 mm and 5.5 ± 0.5 mm ($N = 120$ chirps from the right) for cricket 1 and cricket 2, respectively. The abduction movement initiated 43 and 138 ms after the start of the right chirp for cricket 1 and cricket 2, respectively. Muscle 7, which controls the abduction, showed a marked increase in its activity 90 and 165 ms after the start of the right chirp for cricket 1 and cricket 2, respectively. Abductor muscle 7 was overall more active when the calling song was played ipsilaterally than contralaterally, with 4.4 versus 2.7 spikes/chirp for cricket 1 and 5.4 versus 4.5 spikes/chirp for cricket 2.

In cricket 1, the adduction movement of the antenna precedes the activation of antennal adductor muscle 6, and the abduction movement precedes the activation of antennal abductor muscle

7 in both crickets. An explanation for this phenomenon based on passive forces exerted by the muscles and the scape-pedicel joint is provided in the discussion.

The two crickets responded to the l-r2CP. During a test, the right antenna made small left-right movements and superimposed on these movements were large deflections to the side of the active speaker (Fig. 3.8). The activity of muscle 7 was recorded for both while the activity of muscle 6 was recorded for only one of the crickets (Fig. 3.9). On average, following the chirps from the left, the right antenna was adducted towards the median line and remained adducted until the right chirps were presented. The adduction movement reached a maximum deviation of 3.8 ± 0.3 mm and 5.9 ± 1.2 mm (mean \pm SEM; $N = 45$ repetitions) for cricket 1 and cricket 2, respectively. There was no coupling of the adduction movement with the chirp pattern. This is also reflected in the stimulus histogram of adductor muscle 6, which is not coupled to the chirp pattern even though its spike count

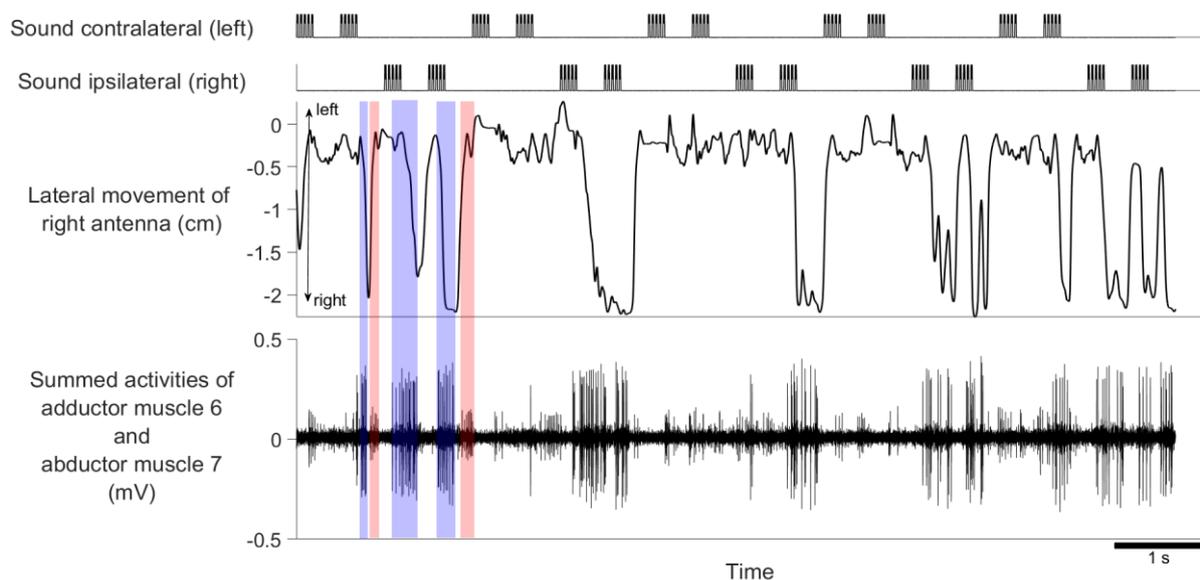


Fig. 3.8 Antennal movement and activity of antennal muscles in a cricket performing phonotaxis towards two speakers playing the l-r2CP. The movement of the right antenna has been measured relative to the median line of the cricket. The small spikes in the EMG belong to antennal adductor muscle 6, whereas the large spikes belong to antennal abductor muscle 7. Activity highlighted in red indicates activity of muscle 6 coupled to the adduction (movement towards the median line/left) of the right antenna and activity highlighted in blue indicates activity of muscle 7 coupled to the abduction (movement away from the median line/right)

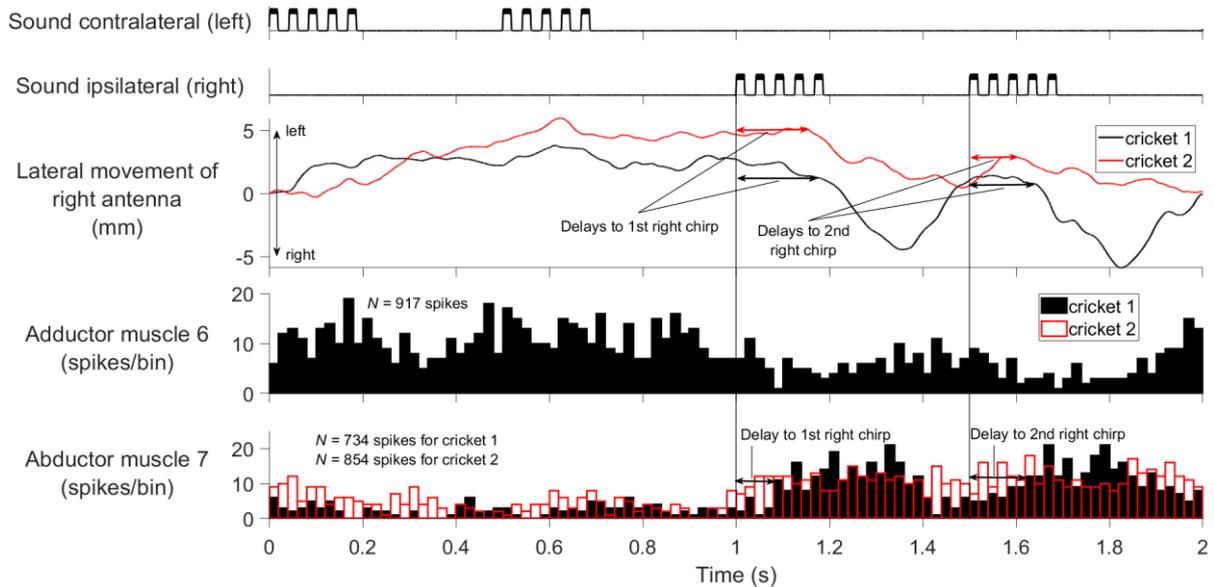


Fig. 3.9 Average antennal movements and PST histograms of antennal muscle activity in two crickets performing phonotaxis towards two speakers playing the l-r2CP ($N = 45$ repetitions). The lateral movement of the antenna has been measured as the deviation from its position at the beginning of the 1st left chrip. The activity of muscle 6 was recorded only in cricket 1. The bin size for the PST histograms is 20 ms.

is higher when sound is played from the contralateral side (608 spikes) than from the ipsilateral side (310 spikes; $N = 45$ repetitions), respectively.

When the calling song is presented from the right (Fig. 3.9), the right antenna responds to both right chirps of the l-r2CP. In cricket 1, the right antenna begins its abduction movement 183 ms after the start of the first right chrip until it reaches 4.4 ± 1.0 mm to the right, then starts moving towards the median line. When the second right chrip is presented, the antenna is abducted again with a delay of 144 ms and it reaches 5.9 ± 1.1 mm to the right. Like the antennal movement that it controls (abduction to the right), also the activity of muscle 7 of cricket 1 is coupled to the chrip pattern when the calling song is played from the right speaker: its spike count shows a marked increase 80ms after the start of the first right chrip and 120 ms after the second one. As with cricket 1, the right antenna of cricket 2 is abducted in response to each of the two right chirps of the l-r2CP, with an average delay of 150 and 116 ms relative to the first and second right chrip, respectively. However, there is no apparent coupling of the activity of muscle 7 of cricket 2 with the chrip pattern, which is

may be related to its more “noisy” antennal movement. Still, muscle 7 of both crickets is more active when sound is played ipsilaterally than contralaterally, with 603 versus 131 (cricket 1, $N = 45$ repetitions) and 613 versus 241 spikes counted (cricket 2, $N = 45$ repetitions).

Finally, cricket 1 responded to the l-r1CP and deflected its right antenna to the right every time the right speaker was activated (Fig. 3.10). The average antennal movement and the stimulus histograms of the antennal muscles during one repetition of the l-r1CP (Fig. 3.11) are in line with what has been reported above. When the left chirp is presented, the antenna moves to the left and reaches a maximum deviation of 2.5 ± 0.3 mm ($N = 19$ repetitions). Adductor muscle 6, which controls the leftwards movement of the right antenna, is more active when the left than when the right chirp is played, with 114 versus 71 spikes counted ($N = 19$ repetitions), respectively. However, the activity of the muscle is not coupled to the chirp pattern. When the right chirp is played, the antenna begins to move to the right 70 ms after the start of the chirp and reaches a maximum deviation of 7.2 ± 1.4 mm. Antennal abductor muscle 7, which pulls the antenna laterally when activated, is exhibiting a marked increase in its spike count 180 ms after the start of the right chirp. Note that the muscle appears to be

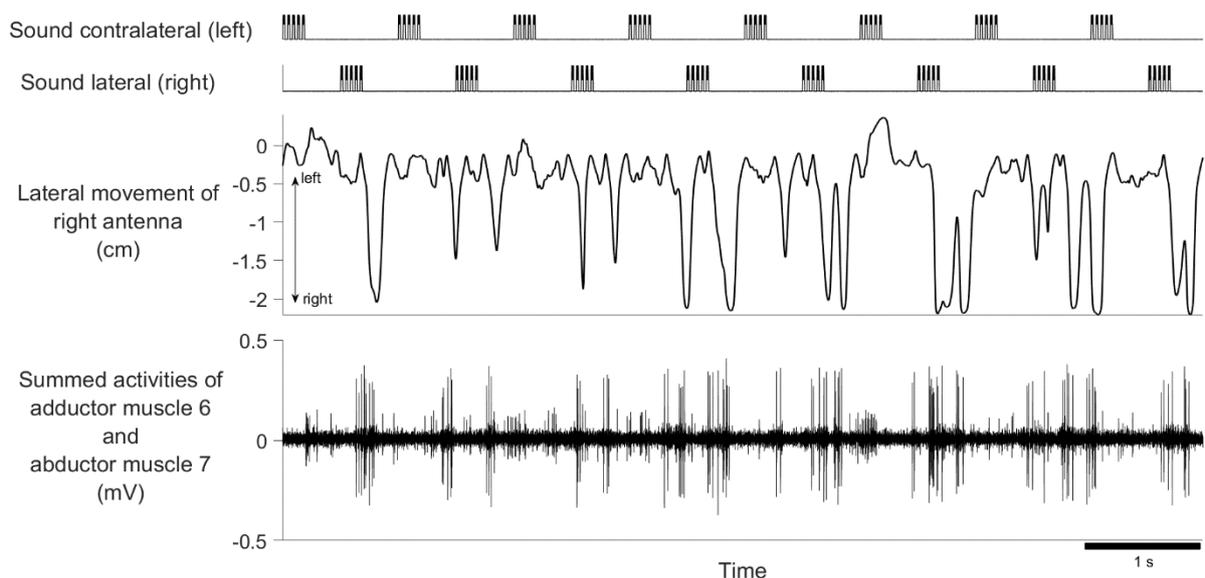


Fig. 3.10 Antennal movement and activity of antennal muscles in a cricket performing phonotaxis towards two speakers playing the l-r1CP. The lateral movement of the right antenna has been measured relative to the median line of the cricket. The small spikes in the EMG belong to antennal adductor muscle 6, whereas the large spikes belong to antennal abductor muscle 7.

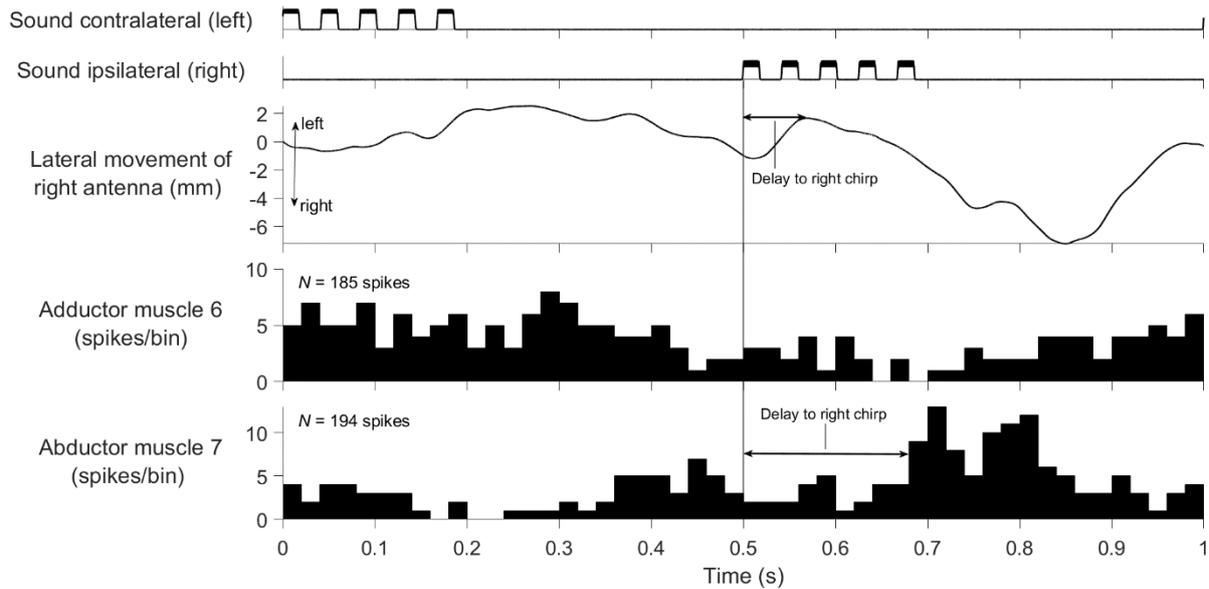


Fig. 3.11 Average antennal movement and PST histograms of antennal muscle activity in one cricket performing phonotaxis towards two speakers playing the l-r1CP ($N = 19$ repetitions). The lateral movement of the antenna has been measured as its deviation from its position at the beginning of the left chirp. The bin size for the stimulus histograms is 20 ms.

activated after the initiation of the antennal movement. Muscle 7 is less active when the ipsilateral (left) than when the contralateral (right) chirp is played, with 59 versus 135 spikes counted ($N = 19$ repetitions), respectively.

Muscle 55

Prosternal muscle 55 consists of two units; both originate at the episternum of the prothorax and insert into the tentorium of the head, with unit *a* inserting into the middle of the hind edge of the tentorial plate, and unit *b* inserting on the dorsal surface of the tentorial plate (Fig. 3.12). Due to the insertion of the muscle in the prothorax, it was a candidate for contribution to the prothoracic steering movement. However, recordings of its activity in walking crickets did not show a correlation with the prothoracic or leg movements. Instead, electrical stimulation of the muscle in a tethered cricket showed that its contraction depresses the head ventrally (Fig. 3.13).

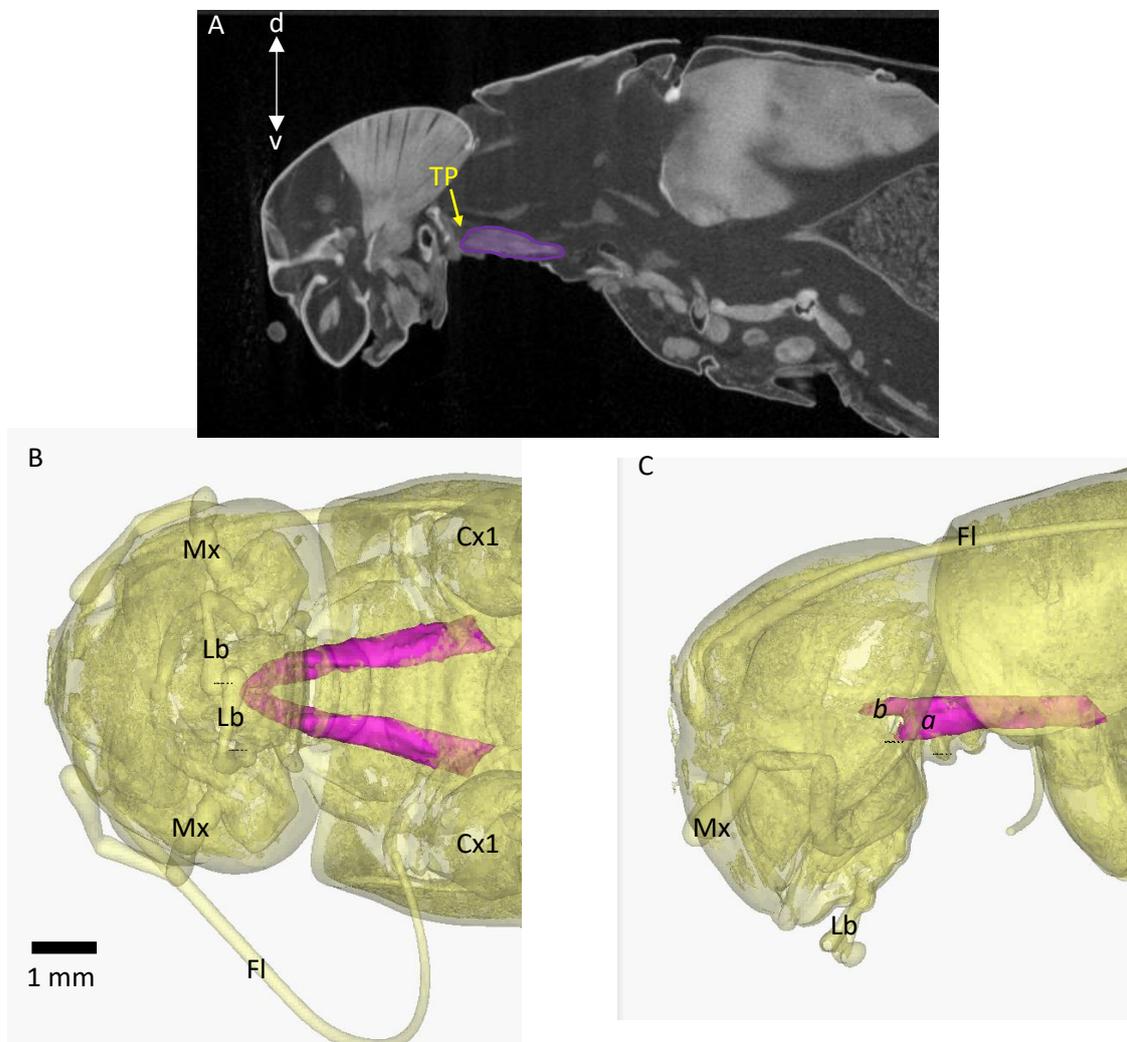


Fig. 3.12 Anatomy of ventral prothoracic muscle 55. (A) CT scan slice with muscle 55 highlighted in purple. (B) Ventral view of 3D model of the head and prothorax with muscle 55 shown in purple. (C) Side view of the 3D model showing the two units (*a* and *b*) of muscle 55. Cx1: prothoracic coxa; Fl: flagellum; Lb: labium; Mx: maxilla; TP: Tentorial Plate; d: dorsal; v: ventral.

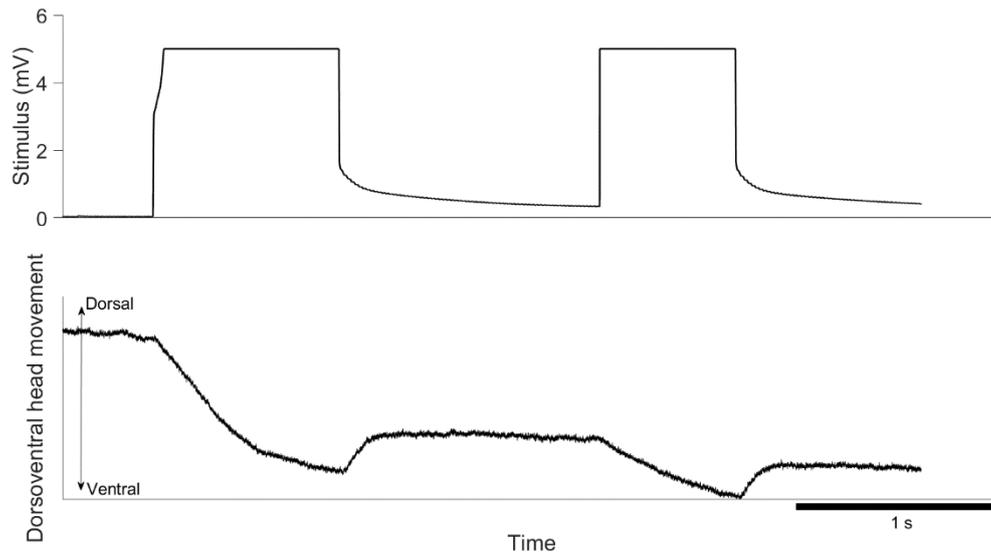


Fig. 3.13 Electrical stimulation of muscle 55 depressed the head ventrally.

Muscle 56

Pronotal muscle 56 resides right underneath the pronotum. It originates at the posterior edge of the pronotum and its other end attaches to the inflexed posterior border of the neck membrane (Fig. 3.14).

In the preliminary tests where the lateral movement of the prothorax and the activity of the left and right muscle 56 were recorded, the animals that performed phonotaxis (9 out of 40 or 22.5%) tilted the prothorax to the direction of the calling song, and the prothorax remained tilted for as long

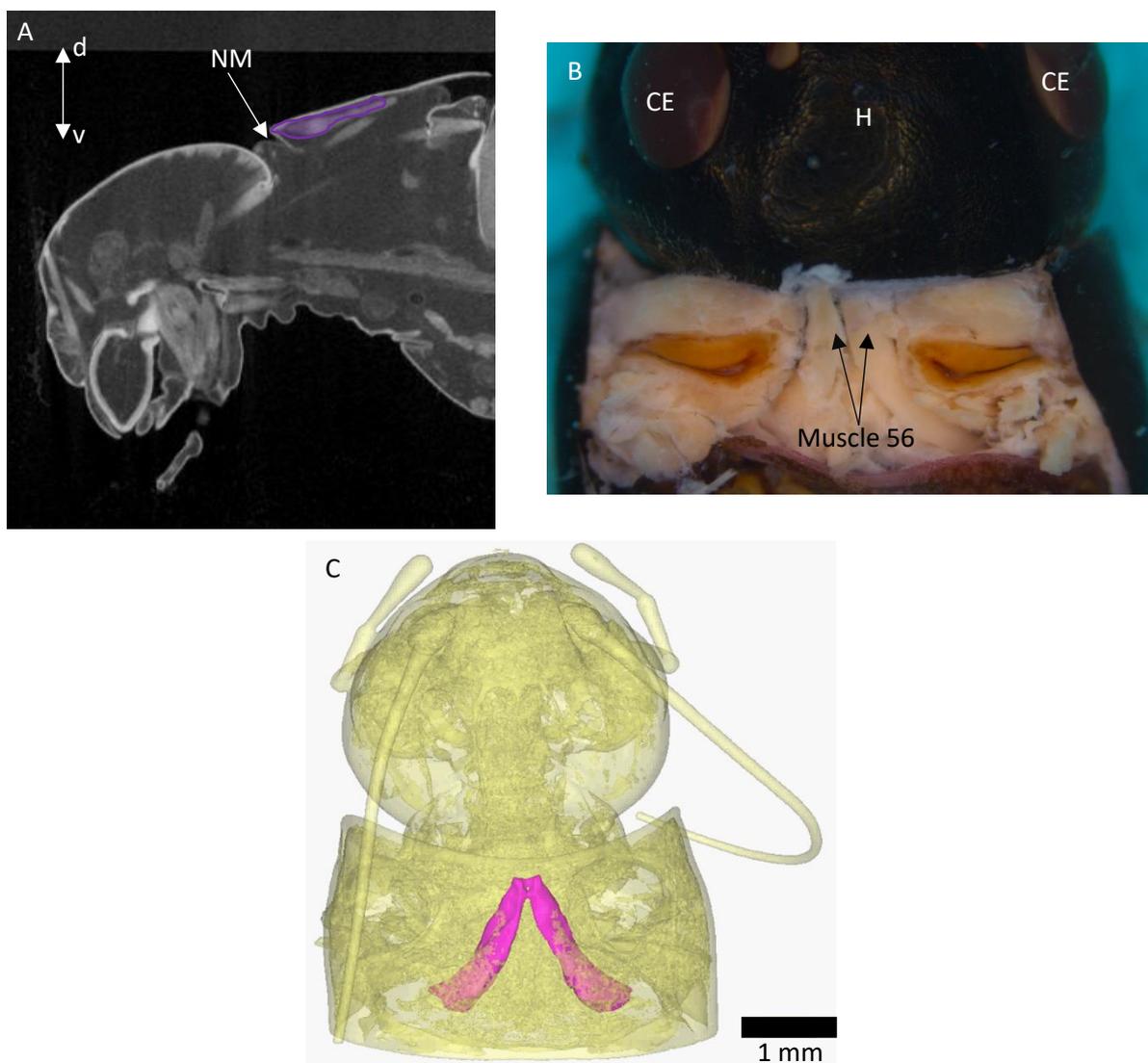


Fig. 3.14 Anatomy of dorsal prothoracic muscle 56. (A) CT slice of head and prothorax with muscle 56 highlighted in purple. (B) Dorsal view of the prothorax and head after the pronotum was removed to reveal the muscle 56 pair. (C) Dorsal view of 3D model of head and prothorax with muscle 56 shown in purple. CE: compound eye; H: head; NM: neck membrane; d: dorsal; v: ventral.

as the calling song was played from that direction (Fig. 3.15A). On top of this auditory-induced movement, the prothorax also moved in accordance to the stepping cycle, and the activity of muscle 56 was coupled to the stepping cycle (Fig. 3.15B). The movement of the prothorax tracked with the optoelectronic advice is in agreement with the one recorded with the high-speed camera in Chapter

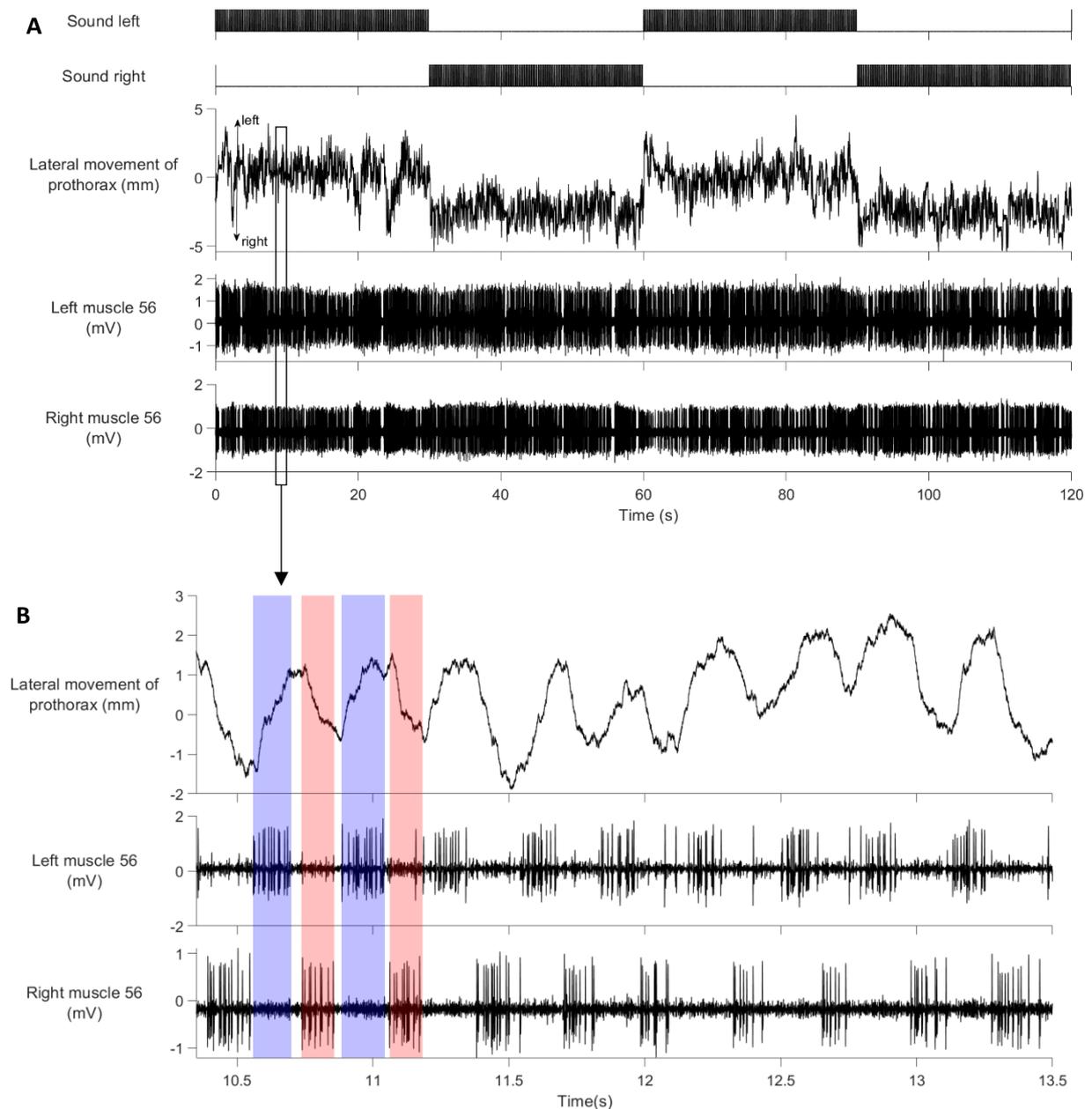


Fig. 3.15 Lateral movement of the prothorax and activity of left and right prothoracic muscle 56 in a cricket performing phonotaxis. **(A)** Response to two speakers playing the calling song for 60 s each. **(B)** The prothorax moves laterally in accordance with the stepping cycle and the activities of the left and right muscle 56 are coupled to this movement (red and blue highlights showcase this coupling). The movement of the prothorax is measured as the deviation from the median line of the cricket.

2. However, the prothoracic movement is less noisy here thanks to the better temporal resolution (0.1 ms versus 3.33 ms).

To study the relationship between the stepping and the activity of muscle 56, sequences where a cricket was walking straight ahead were used, so that the activities of the left or right muscle would not exhibit a potential bias due to the aforementioned auditory-induced tilting of the prothorax. Such walking sequences were obtained from the spontaneous walking of the crickets on the trackball when no calling song was played.

During a stepping cycle the left and right muscle 56 were activated during the leftwards and rightwards movement of the prothorax, respectively (Fig. 3.16). The average stepping cycle had a duration of 352 ± 51 ms (mean \pm S.D., $n = 9$ crickets, $N = 30$ stepping cycles per cricket), and during that time the prothorax deviated 2.1 ± 0.4 mm from left to right. There was a marked increase in the activity of the left muscle 56 19 ± 10 ms before the initiation of the leftwards phase of the prothoracic

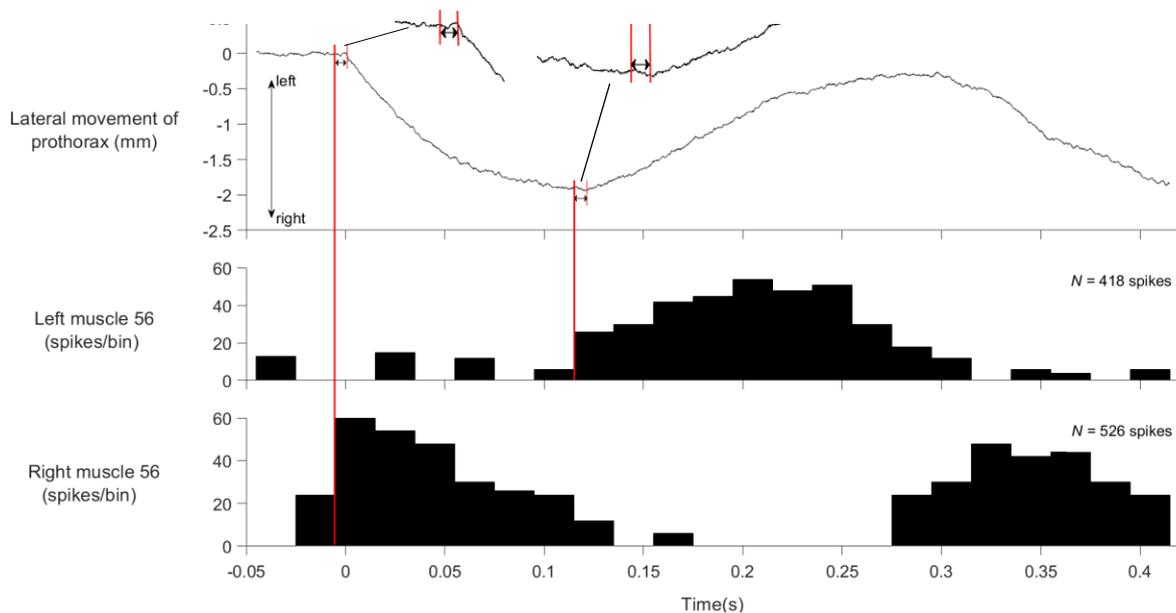


Fig. 3.16 Histograms of the activity of muscle 56 and average movement of the prothorax ($N = 30$ stepping cycles) during a stepping cycle for an individual. The spikes of the muscular activity are binned in 23 bins of 20 ms each. The movement of the prothorax is measured as the deviation from its position at the beginning of the stepping cycle. The double arrows indicate the delay between a marked increase in the activity of the left and right muscles and the initiation of the leftwards and rightwards phase of the stepping cycle respectively.

movement. Accordingly, the activity of the right muscle increased markedly 20 ± 9 ms before the initiation of the rightwards phase of the prothoracic movement. There is no statistical significance between these delays of the leftwards and rightwards movement relative to the activity of muscle 56 (paired sample *t*-test, $P = 0.768$).

The movement of the prothorax and the activity of muscle 56 in response to the calling song were analysed using the l-r2CP and l-r1CP. Out of the 9 crickets that “passed” the preliminary test, 6 responded to the l-r2CP. The crickets steered the prothorax towards the active speaker, and the left and right muscle 56 were activated during both the auditory-induced steering and the stepping cycle (Fig. 3.17). The auditory-induced prothoracic movements are of greater amplitude than those occurring during the stepping cycle, and the bursts of activity of muscle 56 that are coupled to the auditory-induced movements are of longer duration than those coupled to the stepping cycle.

Each muscle 56 of the pair had greater spike count when the calling song was presented ipsilaterally, and their activities were coupled to the movement of the prothorax, which steered towards the active speaker (Fig. 3.18). There is a significant interaction between the spikes counted for the left and right muscle and the speaker that was active (two-way ANOVA, $F_{1,20} = 13.834$,

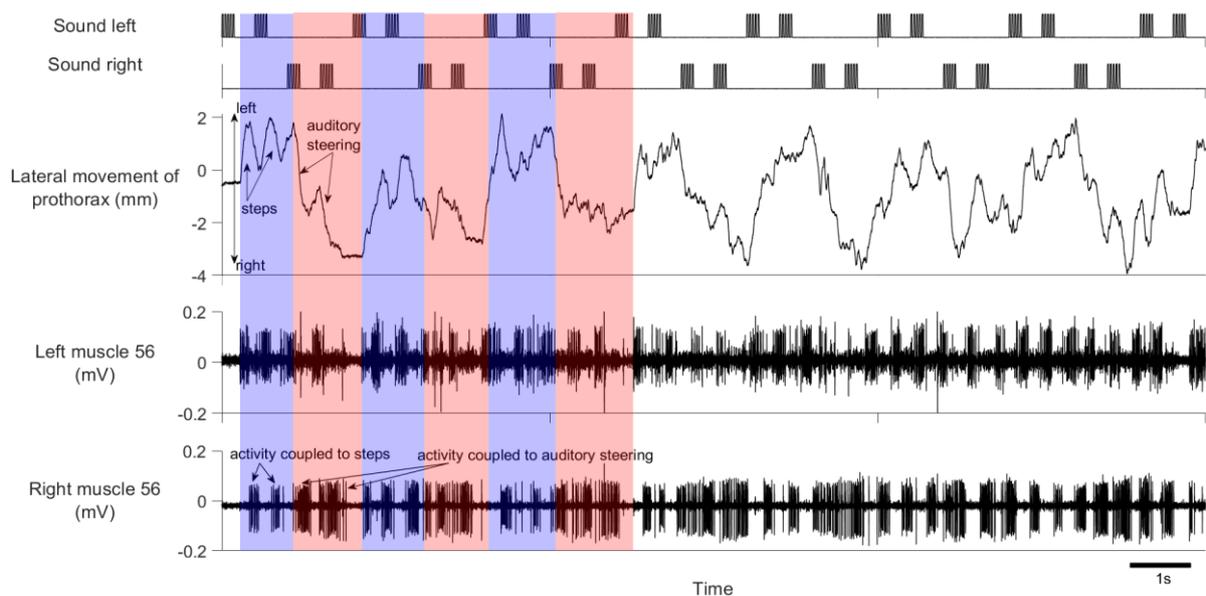


Fig. 3.17 Lateral movement of prothorax and activity of muscle 56 in a cricket performing phonotaxis towards two speakers playing the l-r2CP. Blue and red indicate steering to the left and to the right respectively.

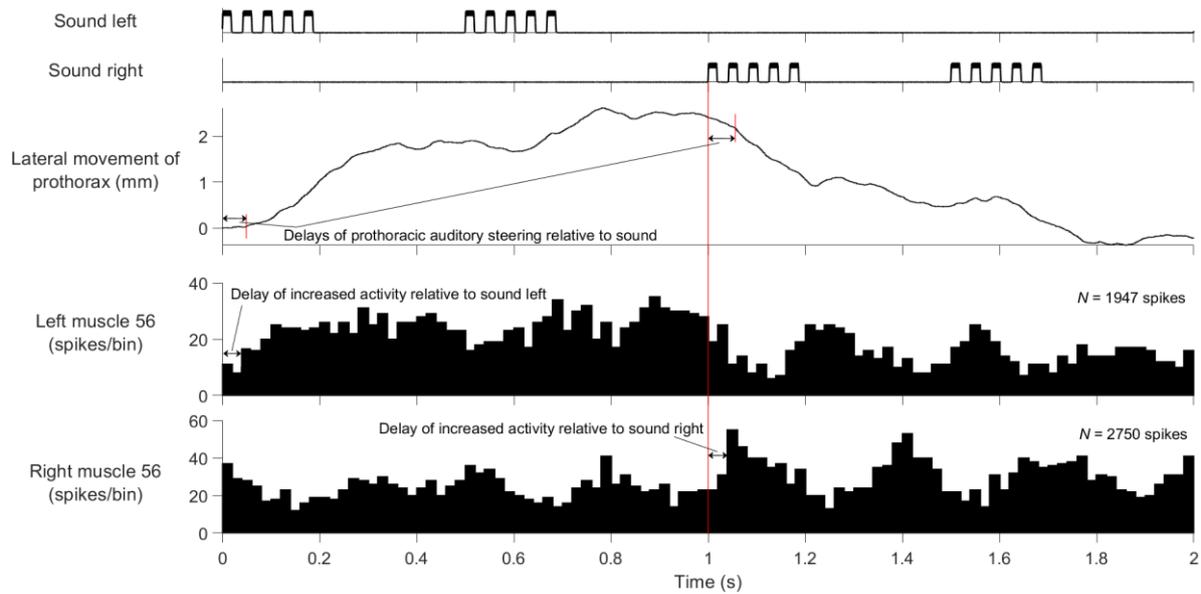


Fig. 3.18 Movement of the prothorax and histograms for muscle 56 in a cricket performing phonotaxis towards two speakers playing the l-r2CP ($N = 30$ repetitions). The spikes of the muscular activity are binned in 100 bins of 20 ms each.

$P < 0.005$). Specifically, when the calling song was playing from the left, the left muscle was more active than the right one, with 47 ± 10 and 39 ± 8 spikes counted per repetition of the l-r2CP (mean \pm S.D., $n = 6$ crickets, $N = 30$ repetitions per cricket), respectively. When two chirps were playing from the right, the spike count per repetition decreased to 35 ± 7 spikes for the left muscle and increased to 49 ± 5 spikes for the right one.

With regard to the temporal relationship between the acoustic stimulus and the activation of muscle 56, the left and right muscle exhibited a marked increase 47 ± 21 ms and 37 ± 8 ms after the start of the first chirp from the left and right respectively. There is no statistical significance between the delays of the left and right muscle 56 (paired sample t -test, $P = 0.296$). The auditory-induced steering of the prothorax initiated 68 ± 29 ms and 50 ± 5 ms after the start of the first chirp from the left and right, respectively. There is no significant difference between the delays of the auditory-induced prothoracic steering to the left and right (paired sample t -test, $P = 0.174$). The maximum left-to-right movement of the prothorax during a repetition of the l-r2CP is 3.0 ± 0.3 mm.

Four out of the 9 crickets that “passed” the preliminary test responded to the l-r1CP. The movement of the prothorax and the activity of muscle 56 during this paradigm are in agreement with the responses reported above, with the prothorax steering towards the active speaker and also moving in accordance to the stepping pattern, and muscle 56 firing during both of these prothoracic movements (Fig. 3.19). No significant effect of the sound direction was found on the spike count of the left and right muscle 56 for this paradigm (Fig. 3.20; two-way ANOVA, $F_{1,12} = 3.53$, $P=0.0849$), however this is likely due to the small number of crickets that responded ($n = 4$ crickets, $N = 60$ repetitions per cricket). The left muscle fired 23 ± 5 spikes per left chirp and 19 ± 4 spikes per right chirp, whereas the right muscle fired 20 ± 3 spikes per left chirp and 24 ± 4 spikes per right chirp. The spike counts of the left and right muscle increased markedly 75 ± 47 ms and 35 ± 10 ms after the start of the left and right chirp respectively. There is no significant difference between the delays of the left and right muscle (paired t -test, $P = 0.161$). The auditory-induced prothoracic steering initiated with a longer delay than the auditory-induced activation of muscle 56. The delay of the prothoracic steering relative to the sound stimulus is 84 ± 52 ms after the start of the left chirp and 48 ± 5 ms after the start of the right chirp. There is no significant difference between the delays of the prothoracic steering to

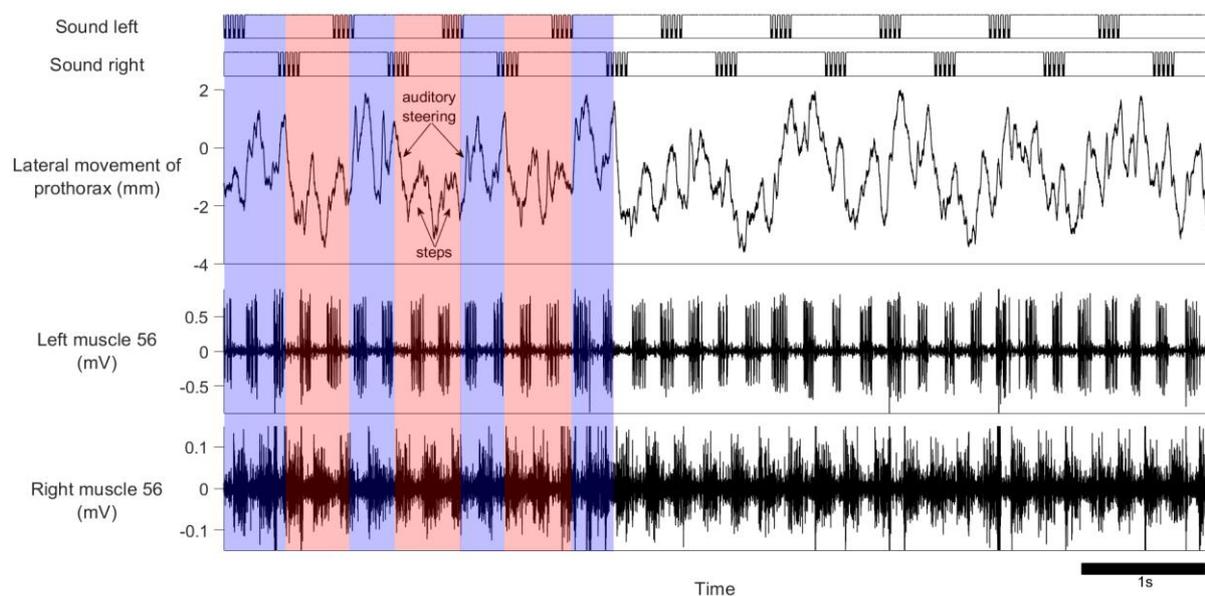


Fig 3.19 Lateral movement of prothorax and activity of muscle 56 in a cricket performing phonotaxis towards two speakers playing the l-r1CP. Blue and red indicate steering to the left and to the right respectively.

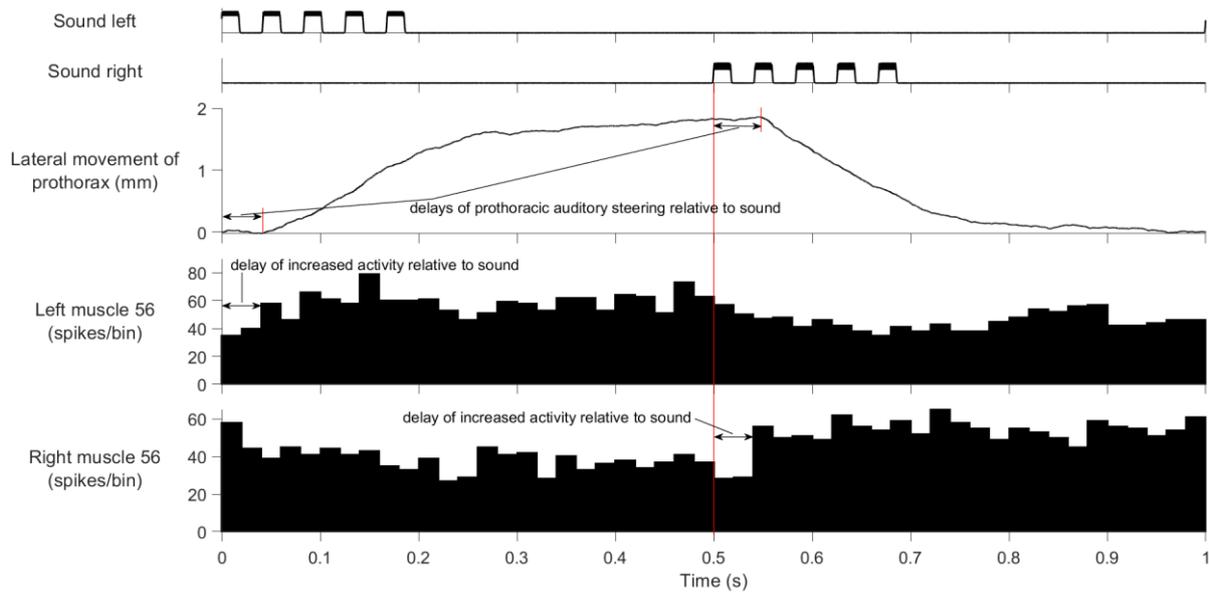


Fig. 3.20 Movement of the prothorax and PST histograms for muscle 56 in a cricket performing phonotaxis towards two speakers playing the l-r1CP ($N = 60$ repetitions). The spikes are binned in 50 bins of 20 ms each.

the left and to the right (paired t -test, $P = 0.224$). The maximum left-to-right deviation of the prothorax during a repetition of the l-r1CP is 1.8 ± 0.2 mm

Muscle 59

Muscle 59 is intersegmental and connects the mesothorax, where it attaches dorsally on the outer angle of the first phragma, with the prothorax, where it attaches ventrally at the base of the furca (Fig. 3.21). Due to its location, it was not feasible to insert wires to stimulate it or record its activity. However, observing the muscle in animals that had its pronotum removed showed that when it contracts, the prothorax rotates relative to mesothorax. Muscle 59 is therefore a rotator of the prothorax.

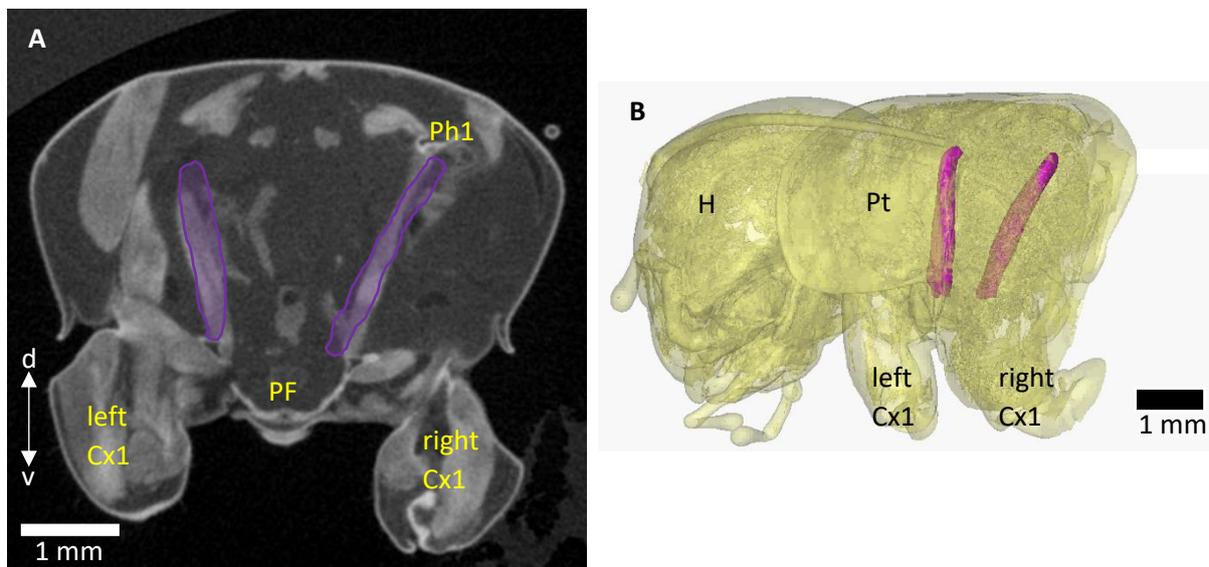


Fig. 3.21 Anatomy of oblique prothoracic muscle 59. (A) CT slice of prothorax with muscle 59 highlighted in purple. (B) side view of 3D model of head and prothorax with muscle 59 shown in purple. Cx1: prothoracic coxa; H: head; PF: prothoracic furca; Ph1: first phragma; Pt: prothorax; d: dorsal; v: ventral.

Muscles 60a and 60b

Both muscles 60a and 60b originate at the first sternal apophysis of the prothorax and cross into the mesothorax, where muscle 60a inserts into the second pleural arm and muscle 60b inserts into the second spina (Fig. 3.22). Based on the the anatomy and attachment points of the muscles, their contraction pulls the prosternite and mesosternite together, causing the prothorax to depress against the mesothorax. Recording of muscle 60a in a tethered cricket showed that its activity is coupled to the rhythmic respiration of the cricket (Fig. 3.23). Specifically, muscle 60a fired every time the prothorax made a small downwards movement during the respiratory cycle, with the muscular activity preceding the start of the downwards movement by 40-60 ms, as based on movement triggered activity histogram (Fig. 3.24). Recording muscle 60b in a tethered cricket showed that its activation is coupled to greater downwards movements of the prothorax than those occurring during the respiration, rendering muscle 60b a depressor of the prothorax (Fig. 3.25).

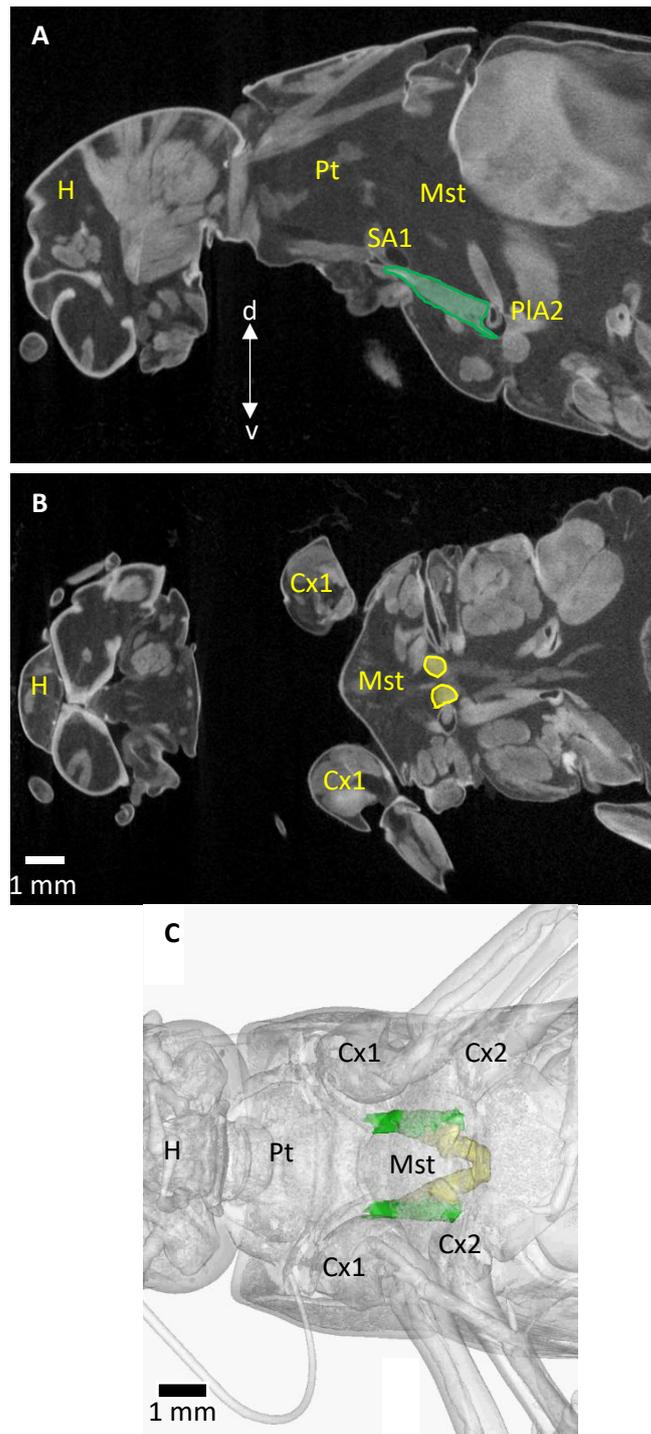


Fig. 3.22 Anatomy of muscles 60a and 60b. (A) CT slice with side view of a cricket with muscle 60a highlighted in green. (B) CT slice with ventral view of a cricket with muscle 60b highlighted in yellow. (C) Ventral view of 3D model with muscles 60a and 60b shown in green and yellow respectively. Cx1: prothoracic coxa; Cx2: mesothoracic coxa; H: head; Mst: mesothorax; SA1: first sternal apophysis; PIA2: second pleural arm; Pt: prothorax; d: dorsal; v: ventral.

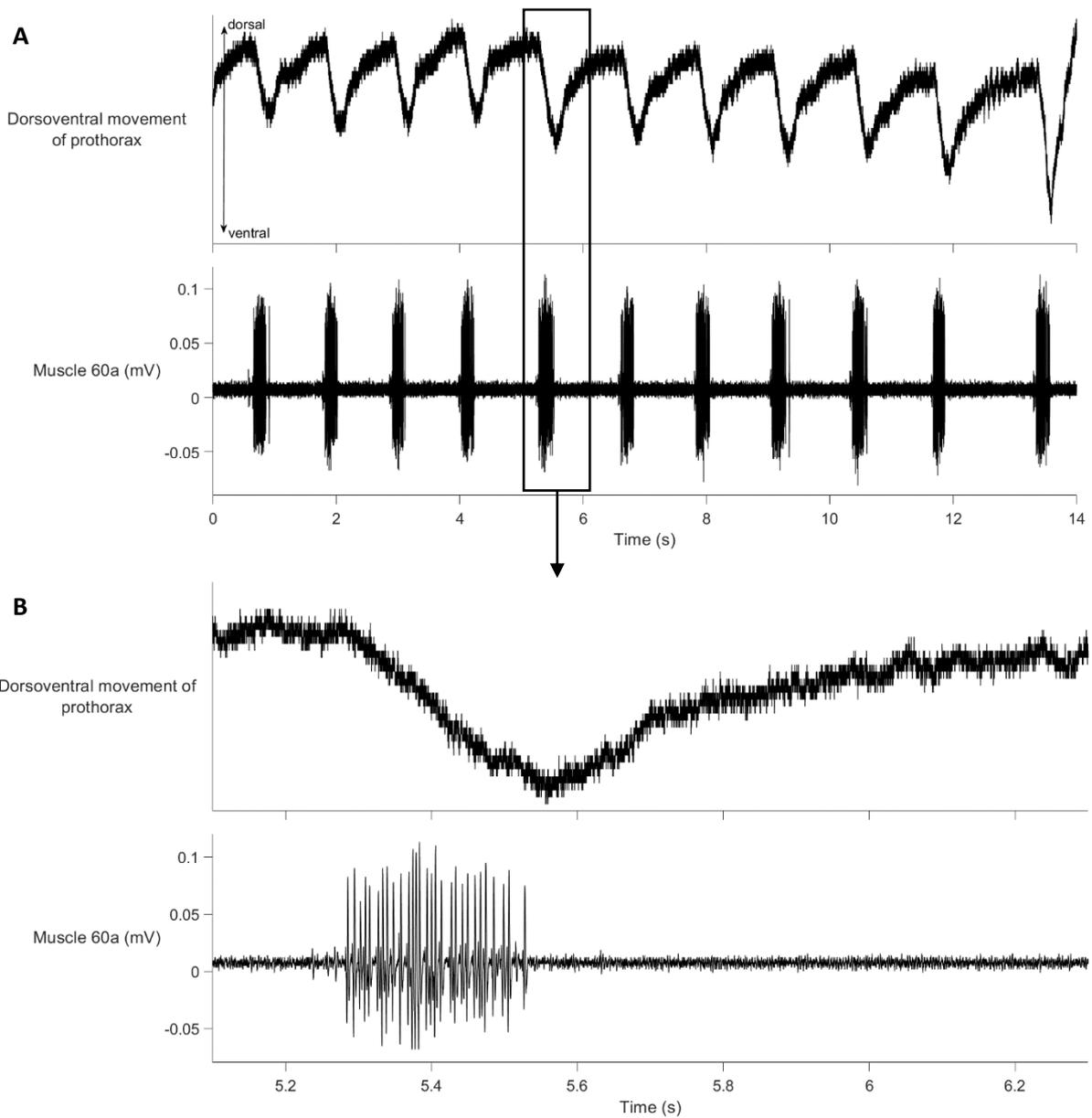


Fig. 3.23 Movement of the prothorax and activity of muscle 60a in a tethered cricket. **(A)** Prothorax moving in accordance with the rhythmic respiratory cycle and activity of muscle 60a coupled to the cycle. **(B)** Close-up of a respiratory cycle and activity of muscle 60a. Note that the low resolution of the movement trace is due to the small-amplitude movement of the prothorax during the respiration.

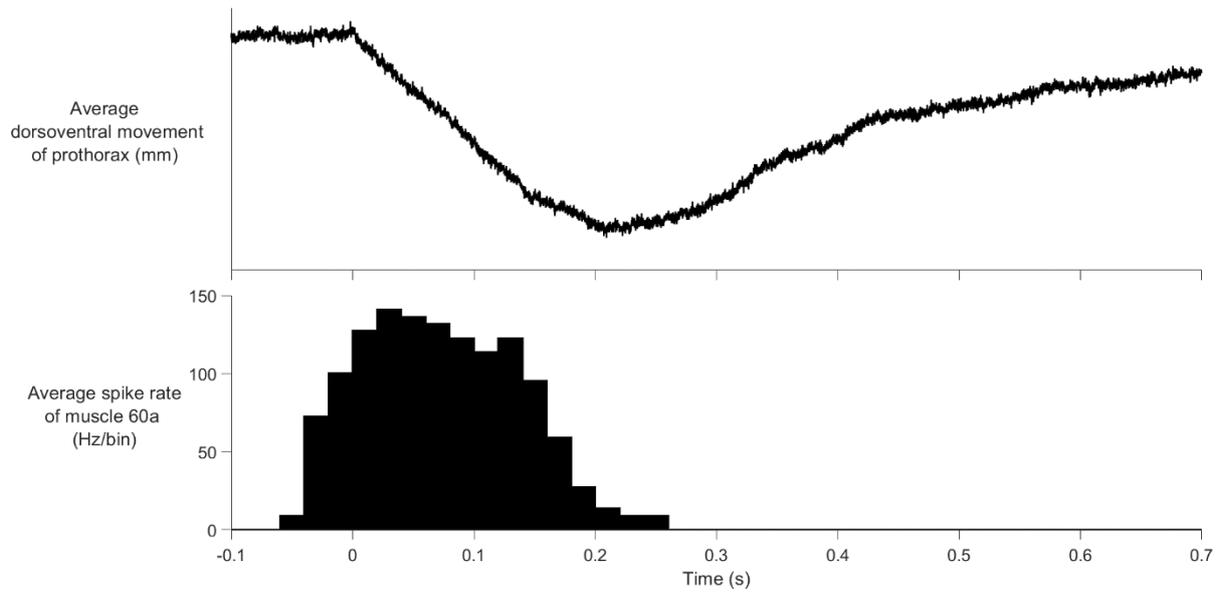


Fig. 3.24. Average dorsoventral movement of the prothorax and average spike rate of muscle 60a ($N=284$ spikes binned in 40 bins of 20 ms each) during a respiratory cycle ($N=11$ respiratory cycles) in a tethered cricket.

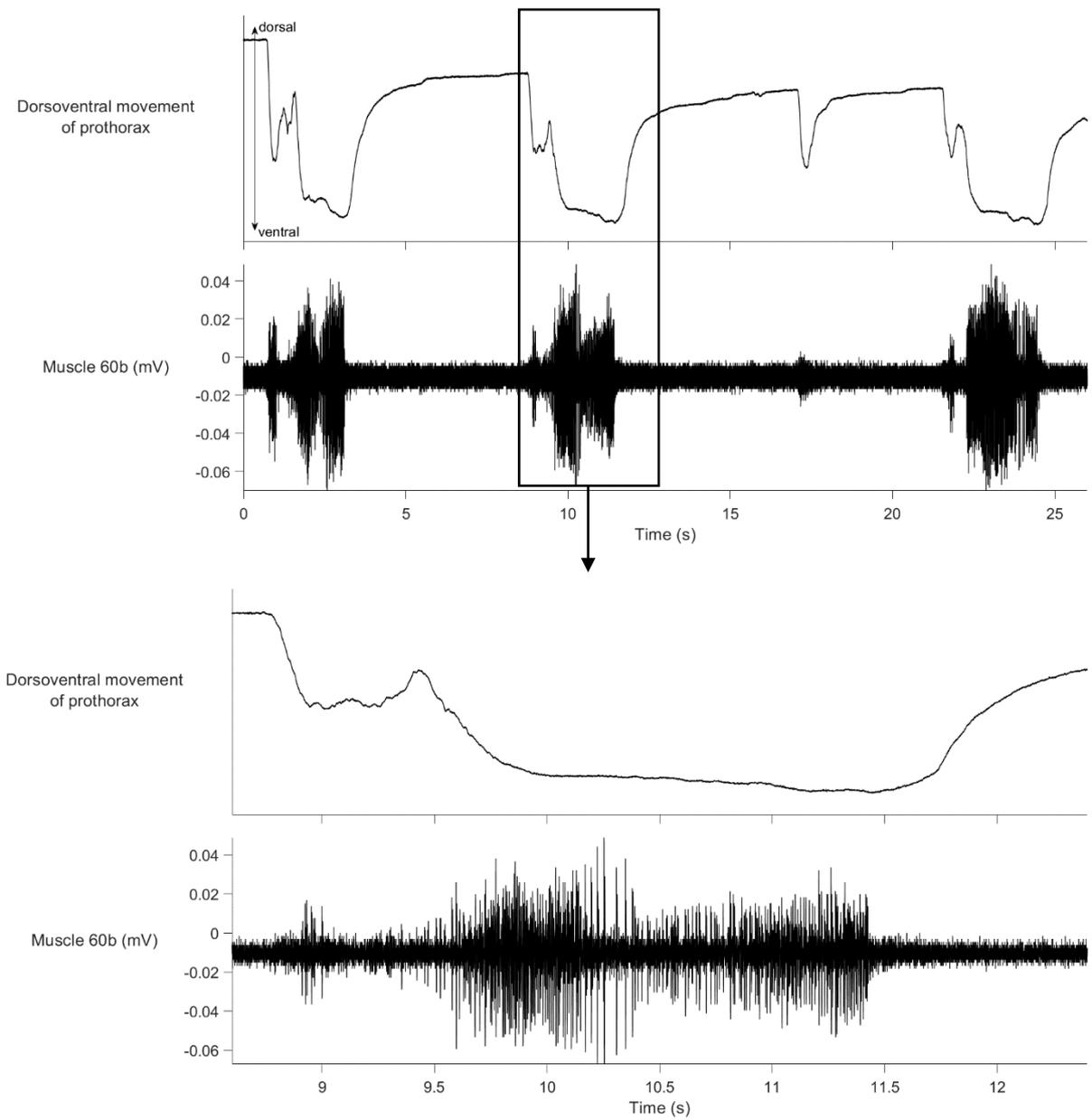


Fig. 3.25 Movement of prothorax and activity of muscle 60b in a tethered cricket.

Muscle 71b1

Muscle 71b1 is an arched muscle that originates at the anterior edge of the pronotum, passes between the pronotum and the pleuron, and terminates at the dorsolateral edge of the prothoracic coxa (Fig. 3.26). Based on its anatomy, its contraction extends the coxa laterally, therefore rendering it an abductor of the coxa. To investigate if muscle 71b1 exhibits auditory responses and how its activity is related to the leg movement, I recorded its activity and tracked the movement of the front femur in a cricket performing phonotaxis (Fig. 3.27A). Muscle 71b1 is activated every time the femur moves during the stepping cycle (Fig. 3.27B). Specifically, the femur ascends during the swing phase of each step and then descends at a slower velocity during the stance phase. The up/down movement of the femur and the activity of the muscle did not exhibit any apparent change when the sound changed direction (Fig. 3.27B). The activity of the muscle exhibits a marked increase approximately 42 ms after the start of the swing phase and 50 ms before the start of the stance phase (Fig. 3.28). Because muscle 71b1 does not directly contribute to the movement of the prothorax and its activity did not exhibit a clear auditory response, its study was not pursued further.

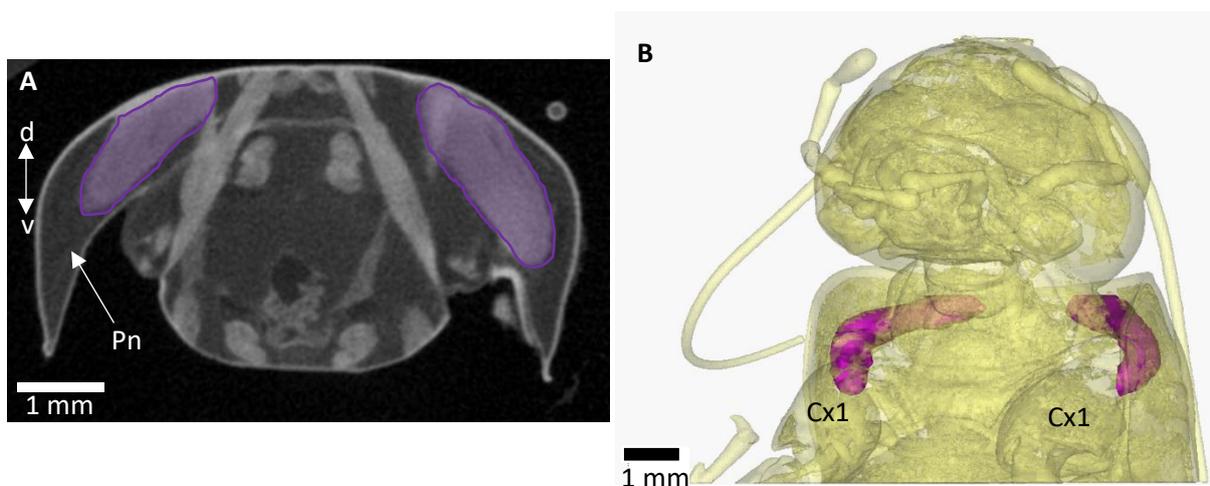


Fig. 3.26 Anatomy of prothoracic muscle 71b1. (A) CT slice of prothorax with muscle 71b1 highlighted in purple. (B) 3D model of head and prothorax with muscle 71b1 shown in purple. Cx1: prothoracic coxa; Pn: pronotum; d: dorsal; v: ventral.

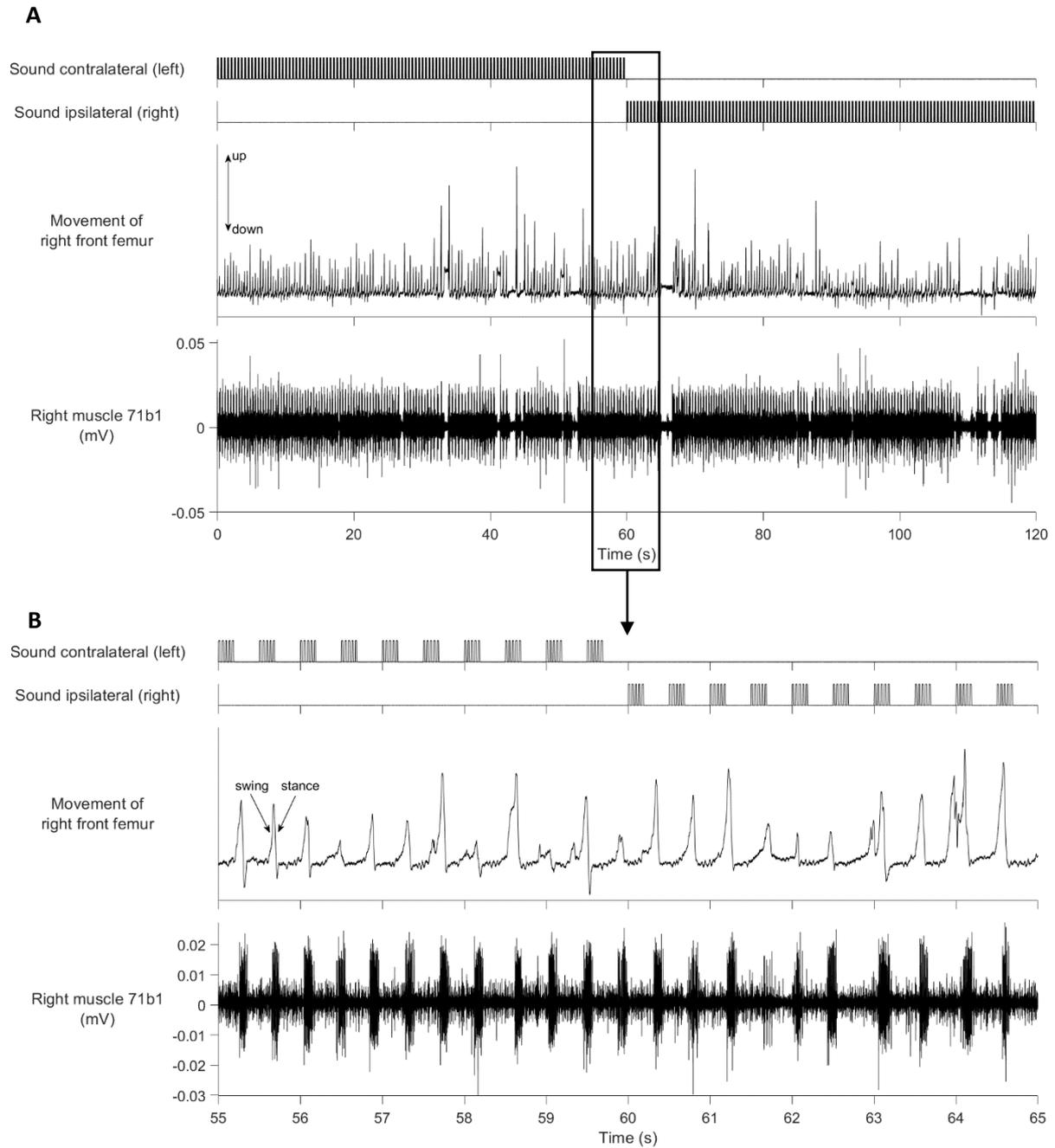


Fig. 3.27 Movement of femur of the front leg and activity of muscle 71b1. **(A)** Responses of a cricket performing phonotaxis towards two speakers playing the calling song for 60s each. **(B)** Close-up around the time point when the sound changes direction.

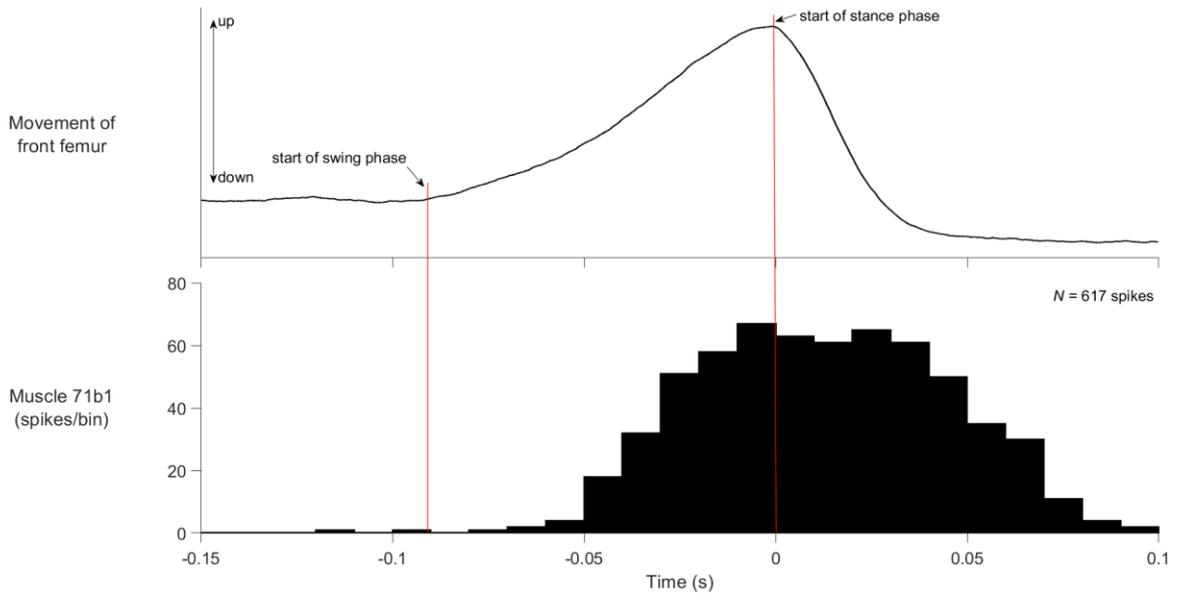


Fig. 3.28 Average femur movement and histogram for muscle 71b1 during one stepping cycle of a cricket ($N = 50$ stepping cycles).

Muscles 87a and 87b

Muscle 87 consists of 2 units, 87a and 87b; both of them originate at the first spina, with 87a inserting into the anterior side of the base of the mesothoracic coxa and 87b inserting into the distal section of the second pleural apophysis. Even though I investigated the function of each unit, the resolution of the CT scan was not sufficient to discriminate between the two (Fig. 3.29).

The location of muscle 87a made it unfeasible to insert wires into it; for this reason, I investigated its function by manually pulling it with forceps. Each pull resulted in the mesothoracic coxa moving downwards and away from the sternum. Therefore, muscle 87a is a depressor of the coxa.

I tested the function of muscle 87b by inserting wires into the left muscle and stimulating it with current. The prothorax moved laterally against the mesothorax every time the stimulus was presented (Fig. 30), therefore muscle 87b potentially contributes to the lateral movement of the prothorax.

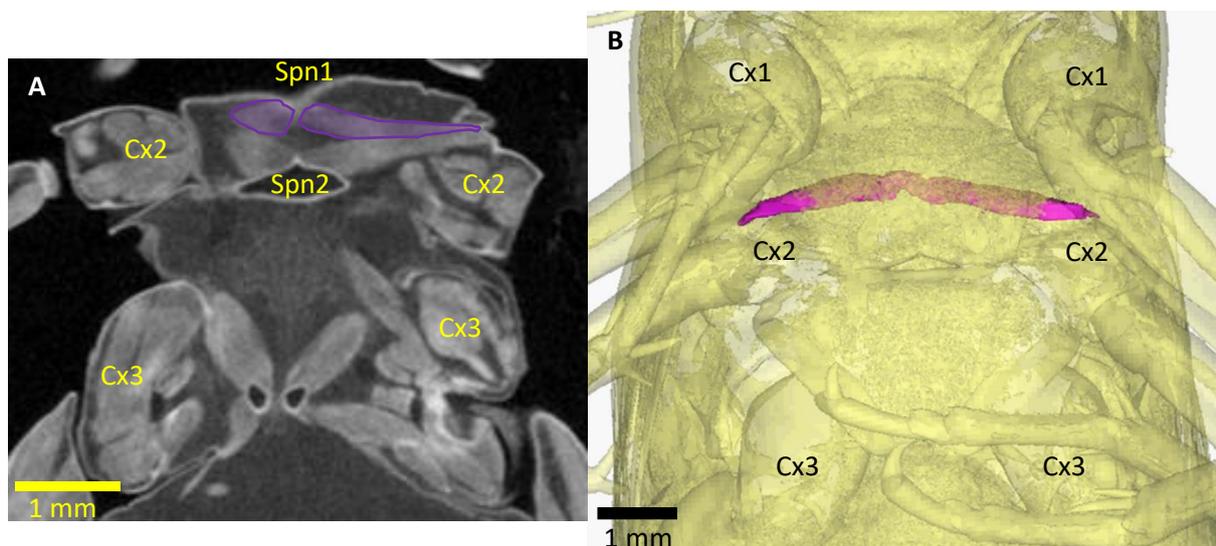


Fig. 3.29 Anatomy of mesosternal muscle 87. (A) CT slice with muscle 87 highlighted in purple. The resolution of the CT scan was not sufficient to discriminate between the two units of the muscle. (B) Ventral view of 3D model with muscle 87 shown in purple. Cx1, Cx2, Cx3: prothoracic, mesothoracic, and metathoracic coxa. Spn1, Spn2: first and second spina.

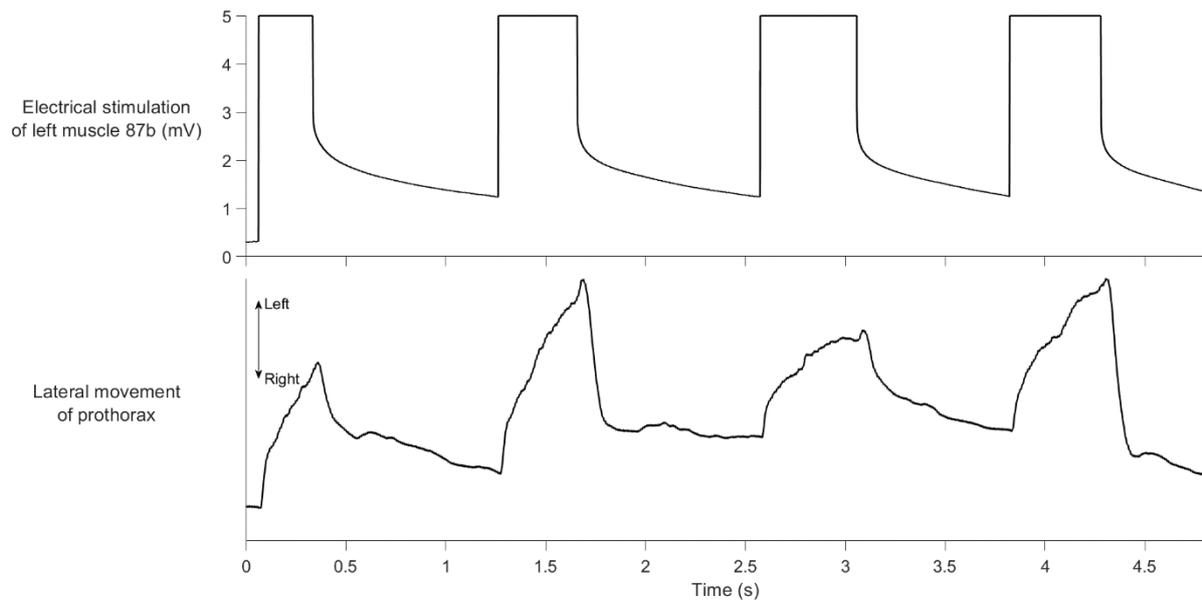


Fig. 3.30 Electrical stimulation of left muscle 87b moves the prothorax laterally.

Discussion

The aim of this study was to identify muscles that contribute to the auditory-induced movements of the antennae and the prothorax. Antennal adductor muscle 6 is activated during the auditory-induced adduction of the antenna in response to the contralateral presentation of the calling song, and antennal abductor muscle 7 is activated during the auditory-induced abduction of the antenna in response to the ipsilateral presentation of the calling song. Of all the thoracic muscles tested, only pronotal muscle 56 exhibited auditory-induced activity in walking animals. Muscles 59 and 87b potentially contribute to the movement of the prothorax, however they could not be tested in walking animals.

Antennal movement and muscles of the scape

The antenna exhibited auditory responses when the calling song played continuously either contralaterally or ipsilaterally (Fig. 3.8). Contralateral stimulation resulted in the adduction of the antenna towards the median line, while ipsilateral stimulation resulted in the lateral abduction of the antenna. The antenna moved less during the adduction than during the abduction, because it can move only slightly past the median line (see Chapter 2) due to the restraints imposed by the biomechanics of the scape-pedicel joint and the muscles inside the scape (Fig. 3.6; also see Honegger

et al., 1990). The spike count of adductor muscle 6 was greater when the calling song played contralaterally than when it played ipsilaterally, however, for one of the crickets there was no obvious marked increase of the muscular activity before or during the adduction movement, possibly due to its small amplitude. Accordingly, the spike count of abductor muscle 7 was greater when the calling song played ipsilaterally than when it played contralaterally, and, in this case, its activity was coupled to the abduction movement for both crickets.

The antenna started to move in response to a chirp with a delay of 53-116 ms when stimulated contralaterally and 43-138 ms when stimulated ipsilaterally (Fig. 3.8). In the high-speed video recordings of Chapter 2, motionless crickets started to move their antennae approximately 180 ms after the calling song started playing following an interval of silence. The longer delay observed in motionless crickets is likely the combination of two reasons. The first one is that the motionless crickets need to recognize the pattern of the calling song, a process that takes place in the brain via a circuit that implements a coincidence detection mechanism (Schöneich, Kostarakos and Hedwig, 2015), before commencing walking. On the other hand, crickets that are continuously presented with the calling song, most likely do not need to constantly recognize the calling song pattern, because pattern recognition modulates phonotaxis transiently (Poulet and Hedwig, 2005). The second reason is that the motionless crickets need to overcome their inertia and generate a motor command for the commencement of walking (review: Bidaye, Bockemühl and Büschges, 2018). Both processes, pattern recognition and the generation of a command for the initiation of walking, add on to the delay of the response of the motionless crickets to the acoustic stimulus.

When the calling song plays constantly either contralaterally or ipsilaterally relative to an antenna, the delay of the muscular activity to individual chirps is 90 ms for adductor muscle 6 and 90-165 ms for abductor muscle 7 (Fig. 3.8). Both during instances of adduction and abduction, the antenna moved before the recording of the corresponding muscular activity. One explanation for this phenomenon is that the recording wires did not pick up the activity of the motoneurons that fire right

before the commencement of the antennal movement. Adductor muscle 6 is innervated by three motoneurons and abductor muscle 7 is innervated by four (Honegger *et al.*, 1990), and, depending on the insertion points of the wires into the muscle, the activity of one or more motoneurons may not be picked up. The alternative – and more likely – explanation is that the antennal movement that precedes the recorded muscular activity is caused by passive forces exerted by the muscles of the scape, or the scape-pedicel joint itself. In general, passive forces can make a substantial and complex contribution to the movement and resting position of the more lightweight appendages (e.g. the antennae), because they can be large enough to overcome the effects of gravity and generate movement in the absence of active muscle contractions (Ache and Matheson, 2012). The passive movement of the antenna was observed in freshly killed crickets, where releasing the antenna after manually adducting or abducting it, resulted in movement towards the resting position. The effects of the passive forces have low variability and therefore the nervous system may be able to “predict” them and adjust the active forces of the muscles accordingly (Ache and Matheson, 2012). In the case of an adducted or abducted antenna, passive forces could initiate movement towards its resting position, and the muscles may start firing as the antenna nears the resting position.

The antenna also exhibited auditory-induced movement towards the side of the active speaker when presented with the l-r2CP and l-r1CP (Fig. 3.9 – 3.12). In line with the findings from the high-speed video recordings of Chapter 2, the auditory-induced antennal movements were superimposed on the rhythmic side-to-side scanning movements (Fig. 3.10 and 3.11). Due to the small amplitude of the auditory-induced adduction, it was difficult to estimate its delay relative to acoustic stimulus, it was however possible to do so for the abduction, whose delay was 80 – 183 ms during the l-r2CP and 70 ms during the l-r1CP. The activity of abductor muscle 7 was coupled to the abduction movement, and its delay relative to the acoustic stimulus was 80 – 120 ms during the l-r2CP and 180 ms during the l-r1CP.

The fact that the antenna and the muscles of the scape (especially abductor muscle 7) exhibit auditory-induced responses to individual chirps either when the calling song is presented constantly from one side (Fig. 3.8) or during the l-r2CP (Fig. 3.10) and l-r1CP (Fig. 3.12) indicates that the motoneurons that innervate the muscles receive auditory input at least on a chirp-to-chirp basis. The antennal motoneurons branch into the dorsal neuropil of the deutocerebrum, whereas the two identified auditory interneurons, AN1 and AN2, ascend from the prothoracic auditory neuropil to the protocerebrum (Moiseff and Hoy, 1983; Schildberger, 1984). Therefore, additional auditory interneurons must be involved for the transfer and processing of the auditory signal from the protocerebrum to the deutocerebrum.

Prothoracic movement and pronotal muscle 56

When the crickets performed phonotaxis, the prothorax steered to the side the calling song was presented from and at the same time made small side-to-side rhythmic movements, which, as shown in Chapter 2, are coupled to the stepping cycle (Fig. 3.16). The activity of the left and right muscle 56 was coupled to the leftwards and rightwards phase of the rhythmic prothoracic movements respectively, with the muscular activity preceding the movement by approximately 20 ms (Fig. 3.17). The temporal resolution, as imposed by the bin size of the stimulus histograms, is also 20 ms, meaning the actual delay of the prothoracic movement relative to the activation of the muscle is ≤ 20 ms. Such delay is in line with what has been previously reported with regards to the delay of the movement of an appendage relative to the activation of the muscle that controls it (e.g. for the leg movement and the activation of the tibia extensor see Baden and Hedwig, 2008).

The activity of muscle 56 was also coupled to the auditory-induced steering of the prothorax, as shown by the responses to the l-r2CP and l-r1CP (Fig. 3.18-3.21). During the l-r2CP the acoustic stimulus induced an increase in the spike count of the muscle with a delay of 37 – 47 ms and the ensuing prothoracic steering with a delay of 50-68 ms (Fig. 3.19). The corresponding delays during the l-r1CP were 35-75 ms and 48-84 ms respectively (Fig. 3.21). Muscle 56 is innervated via three motoneurons that branch into prothoracic nerve 1 (Honegger *et al.*, 1984; muscle 56 is numbered 65

in this study). Two motoneurons have symmetrical arborizations left and right of the midline of the prothoracic ganglion and extend both anteriorly and posteriorly. The third motoneuron has arborizations only ipsilaterally to nerve 1 and does not extend posteriorly as much as the other two. The depth of the arborizations of all motoneurons is between the dorsal median tract and the lateral dorsal tract with projections into the regions of the ventral intermediate tract and the ventral lateral tract. There does not seem to be an overlap between the arborizations of these motoneurons and the ventrally located auditory neuropil, where the primary auditory afferents terminate and where arborizations of interneurons involved in the processing of the auditory information are found (Esch, Huber and Wohlers, 1980; Wohlers and Huber, 1985). Therefore, the auditory input to the motoneurons must be indirect, either via prothoracic interneurons or via a pathway descending from the brain. The motoneurons of the front tibia exhibit a response delay of 35-40 ms to the calling song, which is in the lower range of the delay of muscle 56, and, like the motoneurons of muscle 56, project to the dorsal side of the prothoracic ganglion (Baden and Hedwig, 2008). Based on their delay times, Baden and Hedwig (2008) suggested that the tibia motoneurons likely receive auditory input via a descending pathway, such as descending auditory neurons that respond with a delay of 25–47 ms at the level of the connectives between the suboesophageal and prothoracic ganglia (Staudacher, 2001). Since the motoneurons of muscle 56 have similar projection sites to the tibial motoneurons, and the delay of muscle 56 is equal to or longer than that of the tibial motoneurons, it is also likely that the motoneurons of muscle 56 receive auditory input via a descending pathway as Baden and Hedwig (2008) suggested.

Muscle 59

Due to the location of muscle 59 (Fig. 3.21), it was not feasible to study its function using electrophysiology, however, I observed that contraction of the muscle resulted in the rotation of the prothorax against the mesothorax. During the stepping cycle, apart from tilting towards the front leg in the stance phase (see Chapter 2), the prothorax also rolls towards it (personal observation), therefore muscle 59 could contribute to this movement. Moreover, the prothorax exhibits some roll

during auditory steering (Petrou and Webb, 2012), consequently muscle 59 could contribute to auditory steering as well.

Muscles 60a and 60b

Muscle 60b is activated during the rhythmic respiration of the cricket, and its activity is coupled to the small downward movement of the prothorax occurring during the respiratory cycle (Fig. 3.23 and 3.24). Most insects respire through a system of tubes called tracheas that connect to the air via spiracles that can be actively opened or closed (Chapman, 2013). Mechanisms of respiration include gas exchange (e.g. uptake of oxygen and release of CO₂) through the spiracles (Quinlan and Gibbs, 2006), changes in internal pressure due to hemolymph pumping by the heart or by muscle contraction in the abdomen (Wasserthal, 1996), and body movements that change the shape of tracheal tubes (Weis-Fogh, 1964; Sláma, 1988). In crickets specifically has been reported tracheal compression in the head and thorax via a mechanism of respiration analogous to the inflation and deflation of vertebrate lungs that suggests the involvement of locomotor muscles (Westneat *et al.*, 2003). Based on the insertions and size of muscle 60a (Fig. 3.22), it is likely that it works in synergy with muscle 60b to depress the prothorax downwards (Fig. 3.25) and at the same time contributes to respiration.

Muscle 71b1

Muscle 71b1 had been previously described as a depressor of the trochanter (Furukawa, Tomioka and Yamaguchi, 1983), which is the leg segment between the coxa and the femur. However, according to my dissections and the 3D reconstruction of the muscle from the CT scan, the muscle terminates at the dorsolateral edge of the coxa, without entering it (Fig. 3.26). Its function is most likely the abduction of the coxa away from the body, and it is active both during the swing and stance phase of the stepping cycle (Fig. 3.27 and 3.28).

Chapter 4: Dynamic auditory steering

Abstract

The ability to localize a sound source is essential to the lifestyle of many organisms, whether they are orienting towards a signaling conspecific or evading a predator. Here, I investigated the ability of female field crickets to orient towards a constantly moving speaker playing the male calling song. I ran two sets of experiments, with the speaker moving from 45° right to 45° left and from 10° right to 10° left. In a group of crickets, I fixed the prothorax with wax against the mesothorax to test the effect the immobilization of the prothorax has on their auditory steering. The responses of the crickets with the fixed prothorax did not differ significantly from the crickets that could freely steer the prothorax, even though the crickets with the fixed prothorax understeered towards the more lateral stimulus angles. Overall, the angular resolution of the crickets in the frontal range was 6-11°, which is lower than the 1-2° previously reported for steering towards a static sound source. Phonotaxis towards a moving sound source is more challenging because the binaural cues change constantly, making localization more difficult. In tests with two static speakers, the crickets were able to steer consistently to the correct side when the speakers were positioned at 45° right and 45° left or 5° right and 5° left. Therefore, the angular resolution for a static sound source was at least 5° and further shows that orientation towards a moving sound source is more challenging. Phonotaxis towards 5° was generally “noisier” than at 45°, possibly due to the smaller binaural cues available at 5°. The response delay of the crickets when the presentation of the calling song switched side was longer when the speakers were positioned at 5° than at 45° (164-202 ms versus 136-160 ms), however the difference was not statistically significant.

Introduction

Many organisms have evolved the sense of hearing to use it in the context of communication or to make use of the sound in their environment and enhance survival by collecting information about the acoustic milieu (Fay and Popper, 2000). Regardless of the function of hearing, an animal needs to know where a sound is coming from to allow appropriate motor responses. For example, if hearing is

used for the detection of a potential mate, sound localization will allow the receiver to orient towards a signaling conspecific. Accordingly, if hearing is used for predator detection, the receiver can escape a predator more efficiently if its location is known. Despite the variation among the auditory receivers of vertebrates and invertebrates, sound localization is based on the processing of the interaural time differences (ITD) and the interaural intensity differences (IID). The binaural differences become smaller as the sound incidence angle approaches the longitudinal axis of an animal and are nulled when the sound incidence angle and the longitudinal axis align.

Hearing insects, like most auditory animals, possess bilateral auditory receivers that allow them to localize a sound source via the processing of binaural cues; an exception is the cyclopean ear of the mantis (Yager and Hoy, 1986, 1987, 1989; Yager, May and Fenton, 1990). Due to their small size and short distance between their ears, binaural cues in insects can be very small and hard to detect, at least by human standards. The time it takes for a sound wave to travel the binaural distance (ITD) of an insect can vary from 30 μs for larger insects, such as bush-crickets and locusts, to 1.5 μs for parasitoid flies that have an interaural distance of 500 μm (Robert, 2005). The IID depends on the diffraction of the sound wave around the insect's body, which, according to acoustical theory, becomes significant when the ratio of body size to wavelength is greater than 0.1 (Morse and Ingard, 1968), and is therefore especially relevant for ultrasound. The bodies of some tympanal insects are large enough compared to biologically relevant wavelengths to diffract a soundwave to the extent of creating interaural pressure differences that are sufficient to support directional hearing. Insects that are large enough to diffract a sound wave usually possess ears that function as pure pressure receivers, since the sound pressures on the external sides of the two tympana are sufficiently different for directional hearing (Robert, 2005; Windmill and Jackson, 2016). An example of a pressure receiver is the metathoracic ear of noctuid moths, which is used to detect and evade bats (Roeder, 1998). In the large noctuid moth *Catocala palaeogama*, IIDs for frequencies 30-60 kHz, which are similar to those used by echolocating bats, can be 20-40 dB SPL due to the diffraction of the sound waves around the moth's body (Payne, Roeder and Wallman, 1966). On the other hand, tympanal insects whose size is

too small relative to the wavelength to diffract the sound (e.g. IID due to sound diffraction can be 1-2 dB in a cricket; Michelsen et al., 1994) usually possess ears that function as pressure difference receivers that perceive the pressure difference between the external and internal side of the tympanal membrane (Robert, 2005; Windmill and Jackson, 2016). This requires the sound wave to reach the internal side of the tympanal membrane through a pathway inside the insect. Because the total pressure on the tympanal membrane of a pressure difference receiver is the vectorial sum of all the sound pressures acting on the membrane (e.g. crickets; Michelsen et al., 1994), it is postulated that the internal waves are phase-shifted and amplified or attenuated in an advantageous way as they travel through the internal auditory pathway so that the IID increases (Robert, 2005).

Examples of pressure difference receivers are the ears of crickets and katydids. Crickets possess a pair of tympanal ears on the tibiae of the front legs, with the two ears connected through their acoustic tracheas that transverse the front legs and converge in the midline of the prothorax, where they are separated by one or two membranes called septa (Schmidt and Römer, 2013, 2016). The acoustic trachea has two openings, termed spiracles, one on each lateral side of the prothorax. At the frequency of the calling song (4.5-5.0 kHz), three sound inputs contribute to the directional response of the tympanal oscillation: i) the sound at the external surface of the tympanum, and the sound waves arriving internally through ii) the ipsilateral spiracle and iii) the contralateral spiracle (Michelsen, Popov and Lewis, 1994). At that frequency, the directional sensitivity of the auditory system is maximized, though the IID increases only by approximately 0.33 dB/° for sound incidence angles from 0 to 30°, leading to an IID of about 10 dB at 30° (Michelsen and Löhe, 1995). The medial septum is believed to play an important role in the directional sensitivity by phase-shifting the sound pressure wave from the contralateral spiracle in such way that the directionality of the auditory system is enhanced (Michelsen and Löhe, 1995). In the bush-cricket *Copiphora gorgonensis*, which also possesses a pair of foretibial tympanal ears connected via an acoustic trachea, the narrowing radius of the acoustic trachea reduces the velocity of sound propagation inside it (Jonsson *et al.*, 2016; Veitch *et al.*, 2021). Therefore, the response of each tympanum is shaped by the externally arriving

acoustic signal and its internally arriving delayed version of itself (Veitch *et al.*, 2021). For this reason, these ears are more accurately defined as pressure-time difference receivers. This delay mechanism potentially functions as a delay-line filter for certain frequencies (Veitch *et al.*, 2021).

Due to the difficulty of studying the phonotaxis of female crickets in their natural habitat, most studies on the accuracy of their sound source localization ability have been conducted in the lab. In choice tests using a Y-maze with adjustable 'fork' angle, the calling song was played from one end of the Y-maze, and female *G. bimaculatus* had to choose the correct path (Rheinlaender and Blätgen, 1982). The animals walked randomly to either path for stimulus angles smaller than 15° relative to their longitudinal axis, whereas all the animals chose the direction of stimulation correctly for stimulus angles greater than 25°. The results from the tests with the Y-maze were interpreted as the crickets facing an area of uncertainty within $\pm 25^\circ$ in their frontal range and cannot consistently make correct turns when the calling song is presented from within this area. This notion of low angular resolution in the frontal range persisted in literature and was possibly reinforced by the reported small IID (Michelsen and Löhe, 1995). However, in more recent trackball tests with female *G. bimaculatus* under open-loop conditions, meaning the animals were under constant acoustic conditions because their position relative to the speaker remained constant, they exhibited hyperacute directional orientation and reliably steered towards a speaker playing the calling song, even when the position of the speaker deviated just 1° from their length axis (Schöneich and Hedwig, 2010). Measurements of the animals' tympanal oscillations with laser vibrometry showed that they achieved such hyperacute phonotactic orientation by utilizing a binaural response difference of 0.4 dB/° from 0 to 30° (Schöneich and Hedwig, 2010), similar to the 0.33 dB/° measured before (Michelsen and Löhe, 1995).

In this study, I use a speaker driven by a motor to examine the phonotactic accuracy of female *G. bimaculatus* in response to a novel stimulus paradigm, a sound source moving constantly from 45° left to 45° right and from 10° left to 10° right relative to the crickets' longitudinal axes. A constantly moving sound source is expected to be more challenging to track as compared to a static one because

the animals have limited time to process the auditory information in each speaker position. The experiment will also provide information on the female's directional sensitivity in such a dynamic situation. Based on the experiments of Chapters 2 and 3 showing that the prothorax bends against the mesothorax during auditory steering, I fixed the prothorax against the mesothorax in a group of crickets to test if the lack of prothoracic bending affects auditory steering. In a second set of tests, I used a pair of speakers set at 5° and 45° to test if the response latency of the crickets steering to the calling song is angle dependent. In all tests, the orientation of the crickets was tracked with an open-loop trackball system (Hedwig and Poulet, 2004)

Materials and methods

Crickets and trackball

The rearing and preparation of the crickets for testing on the trackball has been described in Chapter 3, with the difference that no reflective sticker was glued on the crickets. In the animals that belonged to the group with the unrestrained prothorax, the wax used to fix the pin to the back of the animal did not impede the bending of the prothorax against the mesothorax. In contrast, in the group with the fixed prothorax, a drop of wax was applied on the prothoracic-mesothoracic joint to prevent bending of the prothorax against the mesothorax.

Test for the assessment of responsiveness to the calling song and for biased auditory steering

Before each main test, I used the method described in Chapter 3 to assess the responsiveness of a cricket to the synthetic calling song (see Chapter 2 for details) and test for bias to the left or to the right in its auditory steering. In these tests, however, the acoustic stimulus was presented with the moving speaker: the calling song was first presented from +45° (left) for 55 s, the speaker was then muted and moved to -45° (right), where the calling song was presented for another 55 s.

Tests with moving speaker

To investigate female phonotaxis towards a moving sound source, the synthetic calling song was presented to the crickets with a moving speaker (Sinus live, Neo13s, Conrad Electronics, Hirschau, Germany) that was driven by a stepper motor (type 4490H048BK1155, controller MCNL3006S;

Faulhaber GmbH, Schönaich, Germany). The speaker moved in front of the cricket walking on the trackball, and its position was tracked with an angular encoder with a resolution of 0.1° (Absolute Encoder; A2-A-B-E-M-D, US Digital, Vancouver WA 98684, US). The angular velocity was set at $1.1^\circ/\text{s}$; this value was selected after testing the operational parameters of the motor, which is manufactured for much higher velocities, so that it operated robustly, although some “jittering” was still occasionally observed in its movement. The moving speaker followed an arc from -45° (right) to $+45^\circ$ (left) relative to the cricket’s median line, which corresponded to 0° (Fig. 4.1). Each test lasted 830s, that is five speaker oscillations.

A group of crickets was also tested with a speaker movement from 10° left to 10° right. For these tests each speaker oscillation lasted 40 s and the speaker paused for 3 s every time it reached 10° left or 10° right. Each test lasted 440 s, that is 11 speaker oscillations. The aim of the experiment with the narrower angular range was to test if some individuals can follow the speaker movement even at such small angles, which might not be evident when only the mean response of the population is presented. Testing individuals with such extreme performance can determine the angular resolution

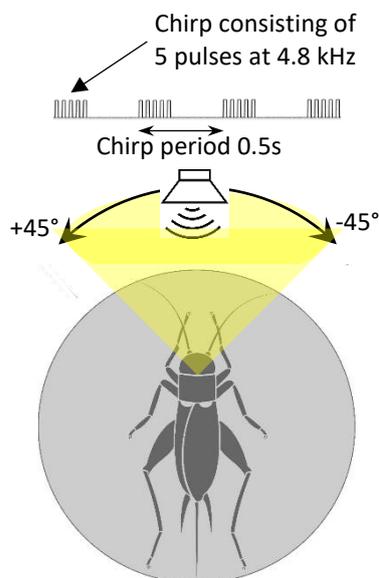


Fig. 4.1 Experimental design for dynamic auditory steering experiments. A speaker playing the calling song moves in front of a cricket walking on a trackball in a range of $\pm 45^\circ$.

that the cricket’s auditory system is capable of in this dynamic stimulus situation. The angular resolution is important when formulating models for the processing of binaural cues or even the general organisation of the auditory-to-motor control system.

Tests with two static sound sources

Two speakers switched every 20 s in the presentation of the calling song, with each test lasting 8 min and presenting 23 switches. First, the speakers were set at -45° and $+45^\circ$, and if an

animal was responsive, the speakers were set at -5° and $+5^\circ$, and the same acoustic stimulus was presented.

Data sampling

Four signals were recorded in each test with the moving speaker: the encoding pulses of the forward/backward (X -channel) and left/right (Y - channel) rotation of the trackball, the angle of the moving speaker, and the sound pattern played by the speaker. For the tests with the two static speakers the four recorded signals were the X - and Y - channels of the trackball, and the sounds played by the two speakers. All signals were sampled at 10 kHz with an A/D board (National Instruments PCI-Mio 16-E-4) controlled by custom software programmed in LabView 5.01.

Processing of data from tests with moving speaker

The walking velocity, the steering velocity, the forward walking, and the lateral deviation of the crickets were derived from the trackball data as described in Chapter 3. Despite using the test for the detection and correction of left/right bias in the auditory steering, some crickets still exhibited a bias during the main test. To reduce the effect of such an asymmetry on the data analysis, a linear curve was fitted to the lateral deviation of the animals that exhibited an asymmetry and then the curve was subtracted from the lateral deviation.

The angle of orientation α of a cricket for each chirp was calculated with:

$$\alpha(i) = \arctan (i)^{-1} \frac{F(i)}{L(i)} \quad (5.1)$$

where F and L are the forward walking and lateral deviation for the duration of chirp i . All angles of orientation corresponding to chirps played at speaker angles within a range of 1° were then averaged (e.g. orientation angles corresponding to chirps played when the speaker was at angles from 0.5° to 1.5° were averaged and the mean was assigned to a speaker angle of 1°).

To obtain the average forward walking and lateral deviation of a cricket over one speaker oscillation, a phase value was assigned to each chirp played by the speaker: a phase of 0 corresponded to the start of an oscillation and a phase of 1 to the end. The phase values were then binned to 91 bins

for the tests from -45° to $+45^\circ$ and to 23 bins for the test from -10° to $+10^\circ$, and the responses belonging to the same bin were averaged for each cricket over all speaker oscillations. In each oscillation the speaker crossed 0° twice: once from left to right and once from right to left.

Statistical analysis for tests with moving speaker

The statistical analysis was done in MATLAB R2019b (Mathworks, UK) and $P < 0.05$ was considered significant. I tested the effect that the cricket group (unrestrained or fixed prothorax) and the angle or phase of the speaker oscillation had on the following parameters:

- i) Forward walking
- ii) Lateral deviation
- iii) Angular orientation
- iv) Angular deviation of cricket from the speaker angle
- v) Forward velocity
- vi) Steering velocity
- vii) Overall velocity

Seven repeated measures models were designed, one for each parameter, where the values of the parameter are the responses, and the cricket group is the predictor variable. For each model, ANOVA and repeated measures ANOVA was performed. ANOVA tested if the values of a parameter differ significantly according to cricket group. Repeated measured ANOVA tested i) for significance in the interaction between cricket group and speaker angle or phase (significance in this case means the two groups have different parameter values at different angles/ phases), and ii) if there is a significant effect of the speaker angle or phase on the parameter. For post-hoc comparisons, I used the Tukey-Kramer test. When the repeated measures ANOVA indicated a significant difference, the Tukey-Kramer test showed at which speaker angle(s)/ phase(s) the parameter's values differ significantly.

Data processing of tests with two static speakers

To estimate the time it took a cricket to cross from one side to the other when the presentation of the calling song switched side, I found the peak and trough in the cricket's average lateral deviation. Because the peak corresponds to the left-to-right crossing and the trough to the right-to-left crossing, their latencies relative to the onset of sound to the right and left respectively show how long it takes the cricket to process the change in sound direction and integrate it into its steering motor response.

Results

Tests with speaker oscillating between 45° left and 45° right

Out of 70 crickets, 35 'passed' the preliminary test – meaning they responded to the calling song – and subsequently responded to the test with the speaker moving within $\pm 45^\circ$. The chirps of the calling song were uniformly distributed across the speaker angles, with slightly less than 2 chirps presented per angle both in the left-to-right and right-to-left part of the speaker oscillation (Fig. 4.2). This ensured that no bias towards specific angles was introduced due to uneven stimulus distribution.

During a test the steering velocity fluctuated, however, steering was generally leftwards when



Fig. 4.2 Distribution of average number of chirps during a speaker oscillation. (A) Left-to-right movement of speaker. (B) Right-to-left movement of speaker ($n=116200$ chirps binned in 91 bins).

the speaker was positioned to the left and rightwards when the speaker was positioned to the right (Fig. 4.3B).

The lateral deviation indicated that the crickets were able to follow the speaker movement and changed walking direction near the zero crossings of the

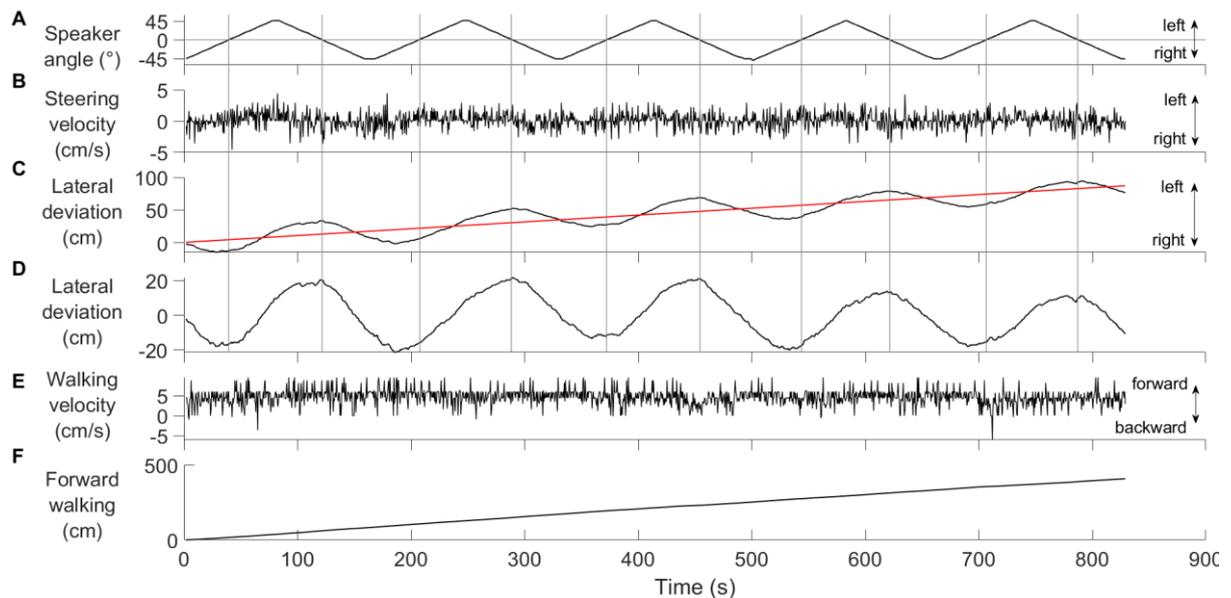


Fig. 4.3 Response of a cricket to a test with the speaker moving from -45° (right) to $+45^\circ$ (left) (A) Position of speaker. The vertical grey lines indicate the zero crossings of the speaker, from left to right and vice versa. (B) Steering velocity of cricket. (C) ‘Biased’ lateral deviation of the cricket obtained by integrating the steering velocity over time. The red line shows linear curve fitted to the lateral deviation ($y = 0.0010x + 0.0077$, $R^2 = 0.8026$). (D) Corrected lateral deviation obtained by subtracting the linear curve from the biased lateral deviation. (E) Walking velocity of cricket. (F) Integration of the walking velocity gives the forward distance walked by the cricket.

speaker (Fig. 4.3C). If a cricket exhibited bias in its lateral deviation, it was corrected by fitting a linear curve to the lateral deviation and then subtracting the linear curve from the lateral deviation (Fig. 4.3D). The walking velocity fluctuated but remained forward moving throughout a test (Fig. 4.3E), which is reflected in the forward walking (Fig. 4.3F).

I tested crickets with an unrestrained prothorax and crickets in which the prothorax and the mesothorax were mechanically coupled. The average steering velocities of both cricket groups increased by absolute value as the speaker moved laterally, although the response was not linear around the zero crossing of the speaker (Fig. 4.4 top), which could be indicative of the crickets’ limitations to orient accurately around the zero crossing. Overall, the speaker angle had a significant effect on the steering velocities (repeated measures ANOVA, $F_{90,2970} = 90.587$, $P < 0.0001$). The crickets with the unrestrained prothorax appear to steer slightly faster than the crickets with the fixed

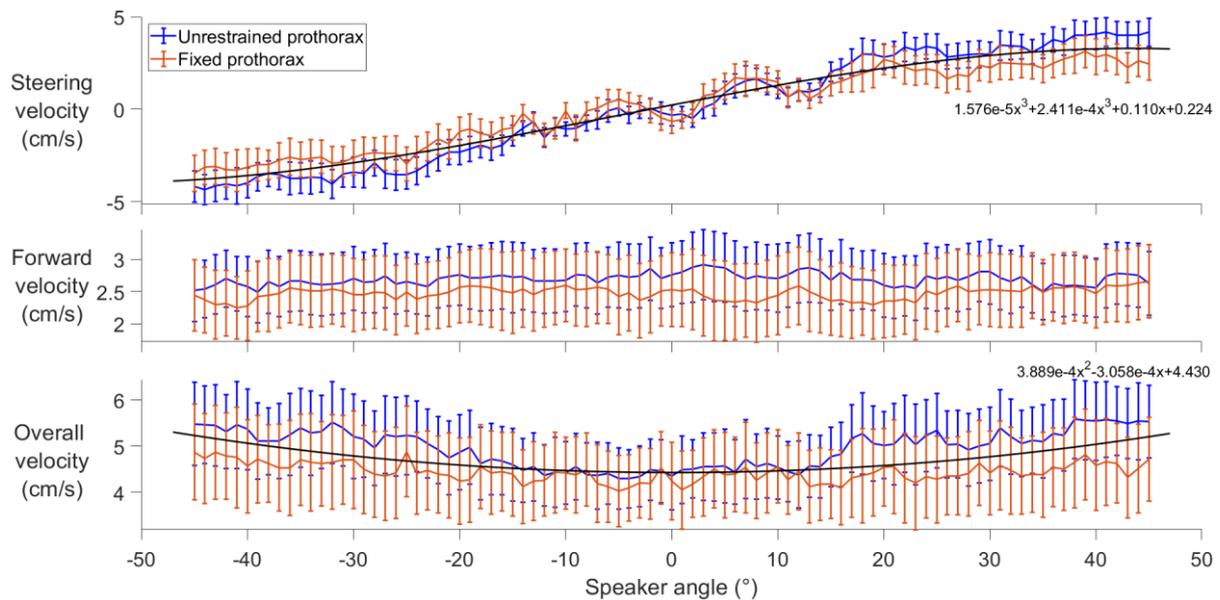


Fig. 4.4 Velocities of crickets for tests with speaker moving from 45° right to 45° left (error bars indicate 95% confidence intervals; $n=20$ crickets with unrestrained prothorax and $n=15$ crickets with fixed prothorax). The polynomials were fitted to the mean response of all crickets, meaning crickets with unrestrained and fixed prothorax were grouped together, because there was no significant difference between the two groups (steering velocity: ANOVA, $F_{1,33}=0.277$, $P=0.602$; forward velocity: ANOVA, $F_{1,33}=0.4026$, $P=0.5301$; overall velocity: ANOVA, $F_{1,33}=0.933$, $P=0.341$).

prothorax, however there is no significant difference between the two groups (ANOVA, $F_{1,33}=0.277$, $P=0.602$).

The forward velocity of the crickets is almost constant with small fluctuations (Fig. 4.4 middle) and does not depend on the position of the speaker (repeated measures ANOVA, $F_{90,2970}=1.492$, $P=0.2305$). Even though the crickets with the unrestrained prothorax generally appear to have greater forward velocity than the crickets with the fixed prothorax, there is no significant difference between the two groups (ANOVA, $F_{1,33}=0.4026$, $P=0.5301$). The mean forward velocity of the crickets with the unrestrained prothorax is 2.70 ± 0.47 cm/s, whereas for crickets with fixed prothorax is 2.48 ± 0.54 cm/s.

Like the steering and forward velocities, the overall velocity (the vectorial sum of the forward and steering velocities) of the crickets with the unrestrained prothorax appears greater than that of the crickets with the fixed prothorax (Fig. 4.4 bottom), however there is no significant difference between the two groups (ANOVA, $F_{1,33}=0.933$, $P=0.341$). The speaker position has a significant effect

on the overall velocity (repeated measures ANOVA, $F_{90,2970} = 5.464$, $P < 0.0001$); specifically, the response follows a parabolic pattern, meaning it increases as the speaker moves laterally and dips around the zero crossing. The parabolic pattern is the result of the forward velocity remaining constant, while the absolute value of the steering velocity increases as the speaker moves laterally.

To calculate the average lateral deviation of a cricket, its lateral deviation at the beginning of each speaker oscillation was reset to 0 cm and then the lateral deviations of all speaker oscillations were averaged. The average lateral deviations of the crickets from both groups show that they were able to follow the trendline of the moving speaker (Fig. 4.5 middle). The phase of the speaker oscillation had a significant effect on the lateral deviation of the animals (repeated measures ANOVA, $F_{90,2970} = 121.235$, $P < 0.0001$), meaning their walking direction depended on the position of the speaker. Even though the animals with fixed prothorax apparently did not turn laterally as much as the ones with the unrestrained prothorax, there is no significant difference between the lateral deviations of the two cricket groups (ANOVA, $F_{1,33} = 0.290$, $P = 0.594$). In theory, if a cricket responded optimally during a single speaker oscillation, the end of its lateral deviation would coincide with the beginning. At the end of a speaker oscillation the total lateral deviation of the animals from their initial position is 2.4 ± 3.1 cm for the unrestrained group, and 1.3 ± 3.5 cm for the group with fixed prothorax. In both groups the end point of the lateral path is not significantly different from the start (Tukey-Kramer test, $P = 0.375$ for unrestrained animals; $P \approx 1$ for animals with fixed prothorax).

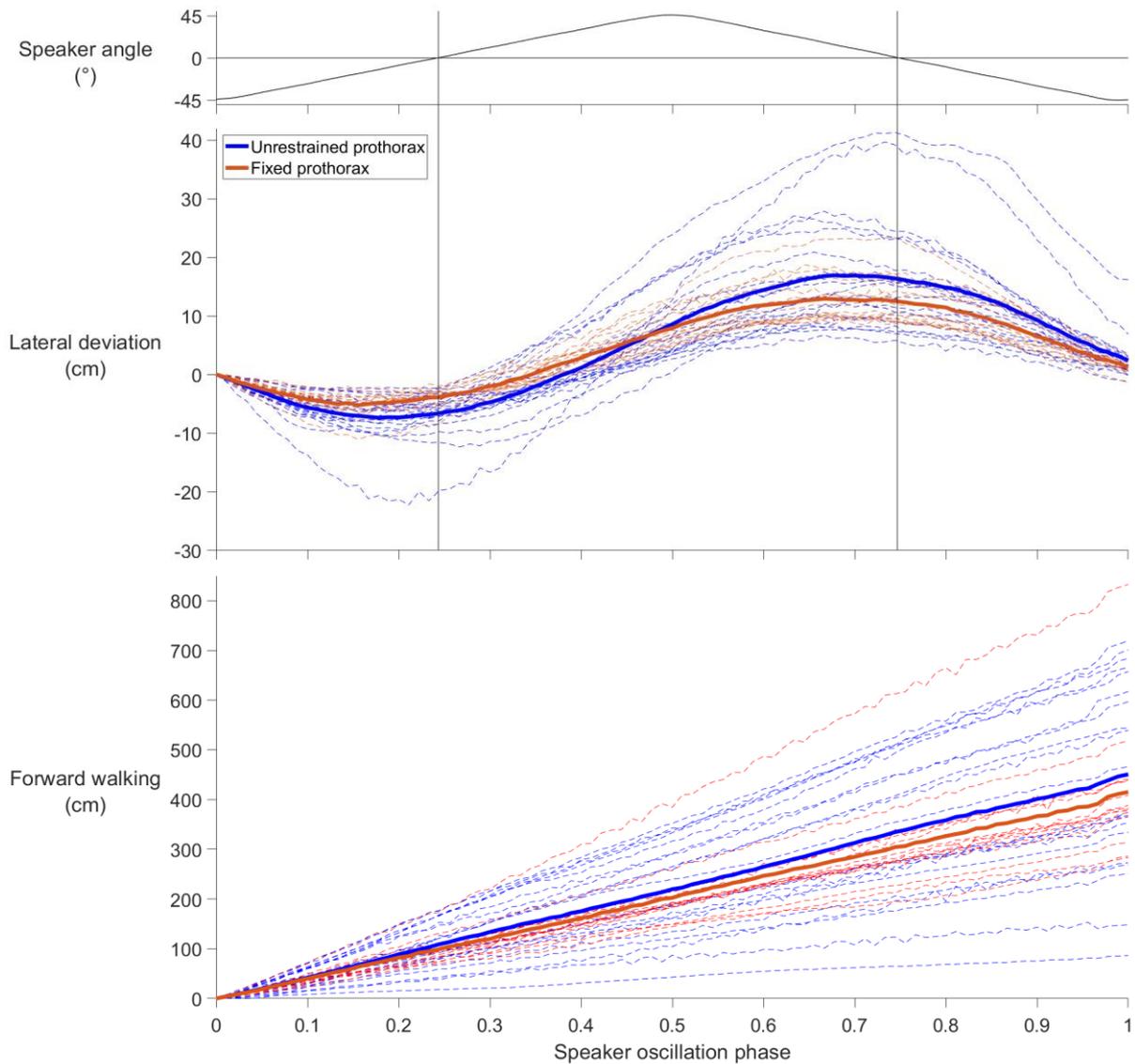


Fig. 4.5 Responses of crickets for one speaker oscillation from 45° right to 45° left. The dashed lines show responses of individual crickets, and the straight lines show the mean response for each cricket group ($n=20$ for unrestrained prothorax group and $n=15$ for fixed prothorax group). The vertical grey lines indicate the zero crossings of the speaker.

As with the lateral deviation, the forward walking of a cricket was reset to 0 cm at the beginning of each speaker oscillation, and then the forward walking paths of all speaker oscillations were averaged. The crickets consistently walked forward, and their forward position depended on the phase of the speaker oscillation (repeated measures ANOVA, $F_{90,2970} = 215.3$, $P < 0.0001$; Fig. 4.5 bottom). On average, during a single speaker oscillation, which lasted 166s, the crickets with the unrestrained prothorax walked forward 450 ± 77 cm ($n=20$; mean \pm 95% C.I.), whereas the animals with

the fixed prothorax walked 415 ± 89 cm ($n=15$). There is no significant difference between the forward distances covered by each cricket group (ANOVA, $F_{1,33}=0.408$, $P=0.409$).

The angle of orientation of the crickets (Fig. 4.6) follows a pattern similar to that of the steering velocity and depends on the angle of the speaker (repeated measures ANOVA, $F_{90,2970}=144.967$, $P<0.0001$). The crickets with the fixed prothorax appear to under-steer compared to the crickets with the unrestrained prothorax, however the responses of the two groups are not significantly different (ANOVA, $F_{1,33}=0.050$, $P=0.825$). To find the angular resolution of the crickets in their frontal range, I used the Tukey-Kramer test to analyze at which speaker angle their orientation angle becomes significantly different from their orientation angle when the speaker is at 0° . Due to an asymmetry in the response of the crickets, their angular resolution is 11° to the right and 6° to the left.

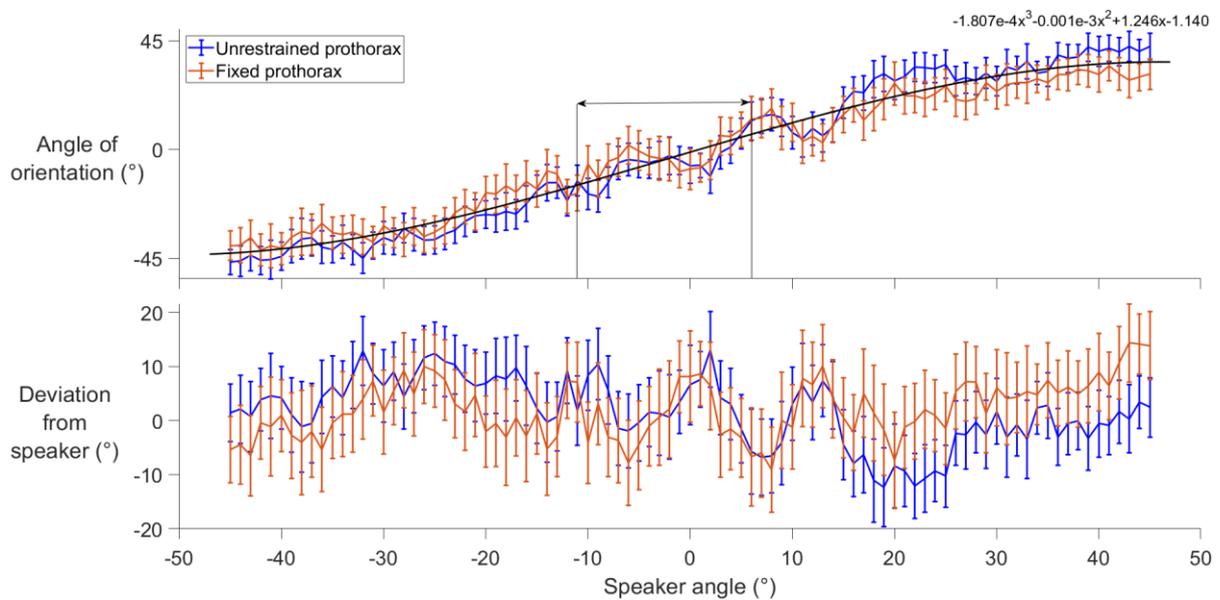


Fig. 4.6 Angle of orientation and deviation angle of crickets from the speaker angle (error bars indicate 95% C.I.; $n=20$ crickets with unrestrained prothorax and $n=15$ crickets with fixed prothorax). The double arrow shows the angular resolution of the animals in the frontal range. The 2nd degree polynomial was fitted to the mean response of all crickets, because there is no significant different between the angles of orientation of the two groups (ANOVA, $F_{1,33}=0.050$, $P=0.825$).

Table 4.1 Synopsis of results from statistical analysis of repeated measures models. The tests show the effects on the parameters of the first column. Pink color indicates statistical significance.

Forward walking	Speaker phase (repeated measures ANOVA, $F_{90,2970}=215.304$, $P<0.0001$)
	Cricket group X speaker phase interaction (repeated measures ANOVA, $F_{90,2970}=0.476$, $P\approx 1$)
	Cricket group (ANOVA, $F_{1,33}=0.409$, $P=0.527$)
Lateral deviation	Speaker phase (Repeated measures ANOVA, $F_{90,2970}=121.235$, $P<0.0001$)
	Cricket group X speaker phase interaction (repeated measures ANOVA, $F_{90,2970}=3.207$, $P<0.0001$)
	Cricket group (ANOVA, $F_{1,33}=0.29$, $P=0.5939$)
Forward velocity	Speaker angle (repeated measures ANOVA, $F_{90,2970}=1.492$, $P=0.2305$)
	Cricket group X speaker angle interaction (repeated measures ANOVA, $F_{90,2970}=1.136$, $P=0.182$)
	Cricket group (ANOVA, $F_{1,33}=0.4026$, $P=0.5301$)
Steering velocity	Speaker angle (repeated measures ANOVA, $F_{90,2970} = 90.587$, $P<0.0001$)
	Cricket group X speaker angle interaction (repeated measures ANOVA, $F_{90,2970}=2.742$, $P<0.0001$)
	Cricket group (ANOVA, $F_{1,33}=0.277$, $P=0.602$)
Overall velocity	Speaker angle (repeated measures ANOVA, $F_{90,2970} = 5.464$, $P<0.0001$)
	Cricket group X speaker angle interaction (repeated measures ANOVA, $F_{90,2970}=1.681$, $P=0.0001$)
	Cricket group (ANOVA, $F_{1,33}=0.933$, $P=0.341$)
Angle of orientation	Speaker angle (repeated measures ANOVA, $F_{90,2970}=144.967$, $P<0.0001$)
	Cricket group X speaker angle interaction (repeated measures ANOVA, $F_{90,2970}=2.202$, $P<0.0001$)

	Cricket group (ANOVA, $F_{1,33}=0.050$, $P=0.825$)
Deviation from speaker angle	Speaker angle (repeated measures ANOVA, $F_{90,2970}=4.177$, $P<0.0001$)
	Cricket group X speaker angle interaction (repeated measures ANOVA, $F_{90,2970}=2.202$, $P<0.0001$)
	Cricket group (ANOVA, $F_{1,33}=0.051$, $P=0.823$)

Tests with speaker oscillating between 10° left and 10° right

Out of the 26 crickets tested, 4 performed phonotaxis towards the speaker when it oscillated from -10° to +10°. The crickets that responded followed the speaker movement for the whole duration of the test and changed their walking direction every time the speaker crossed the midline (Fig. 4.7). As expected, the absolute value of the steering velocity increased as the speaker moved laterally (Fig. 4.8 top), and the effect of the speaker position on the steering velocity was significant (repeated measures ANOVA, $F_{20,60}=10.835$, $P<0.0001$). Both the forward and overall velocities remained almost constant during the speaker movement, with mean values 4.56 ± 2.70 cm/s and 4.57 ± 2.70 cm/s ($n=4$ crickets; mean \pm 95% C.I.) respectively. The forward and overall velocities are almost identical due to the lower

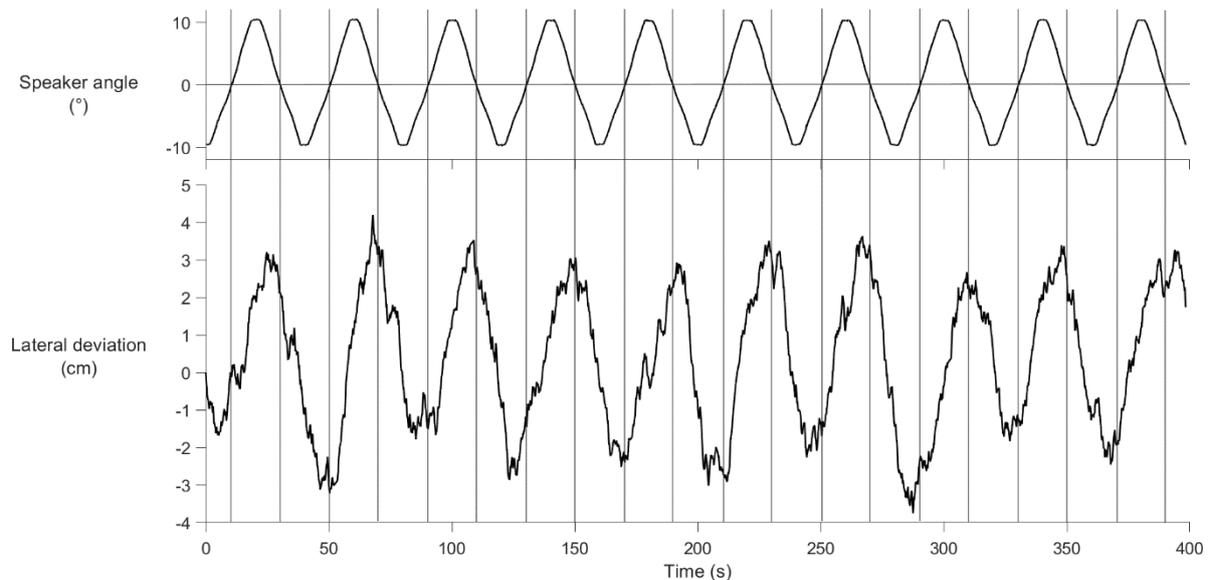


Fig. 4.7 Lateral deviation of a cricket responding to the speaker oscillating between 10° right and 10° left. Note the change in walking direction when the speaker crosses the midline.

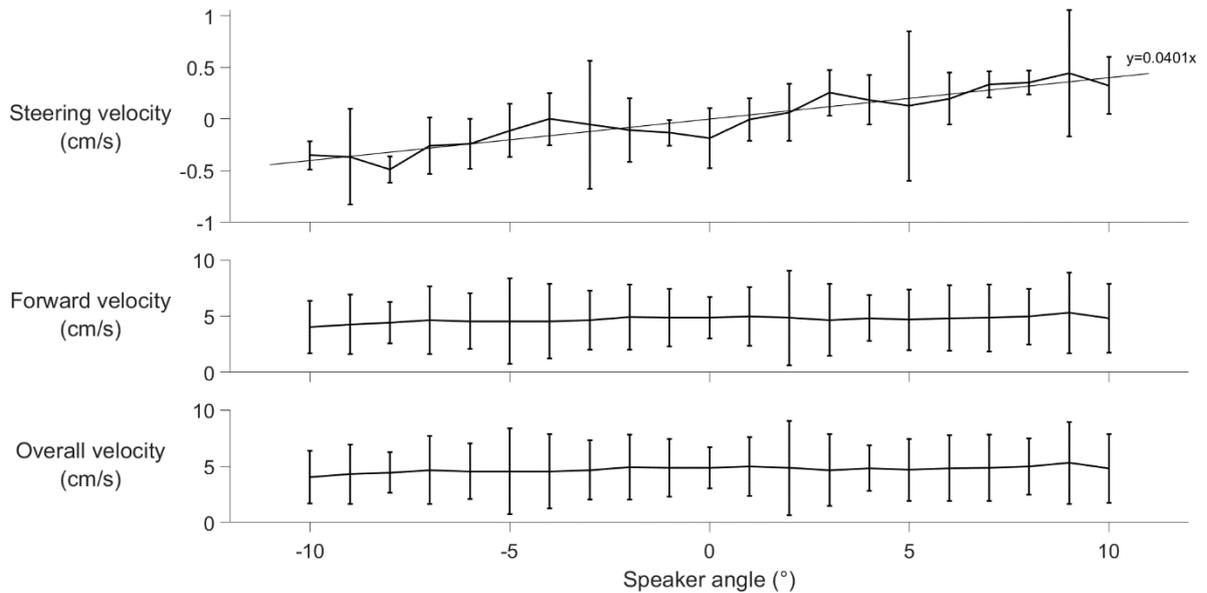


Fig. 4.8 Velocities of crickets for tests with the speaker moving from 10° right to 10° left (error bars indicate 95% confidence intervals, $n = 4$ crickets). The speaker angle has a significant effect on the steering velocity (repeated measures ANOVA, $F_{20,60}=10.835$, $P<0.0001$) but not the forward and overall velocities (repeated measures ANOVA; forward velocity: $F_{20,60}=1.326$, $P=0.2188$; overall velocity: $F_{20,60}=1.291$, $P=0.2397$).

steering velocity compared to the forward velocity; therefore, the forward velocity contributes more to the overall velocity. There was no effect of the speaker position on the forward and overall velocities (repeated measures ANOVA; forward velocity: $F_{20,60}=1.326$, $P=0.2188$; overall velocity, $F_{20,60}=1.291$, $P=0.2397$).

The average lateral deviation for one speaker oscillation indicates that the crickets were generally able to follow the speaker movement, even though the lateral deviation is 'flat' around the right-to-left zero crossing (Fig. 4.9 middle). The 'flat' lateral deviation means that the crickets walked straight ahead around that point instead of steering towards the smaller angles in their frontal range, possibly because they could not resolve the smaller angles. By the end of a speaker oscillation the lateral position of the crickets was 0.4 ± 0.7 cm, which did not differ significantly from their starting position (Tukey-Kramer test, $P=0.966$), due to the symmetry of the speaker oscillation. Overall, their lateral deviation depended on the phase of the speaker oscillation (repeated measures ANOVA, $F_{22,66}=35.945$, $P<0.0001$).

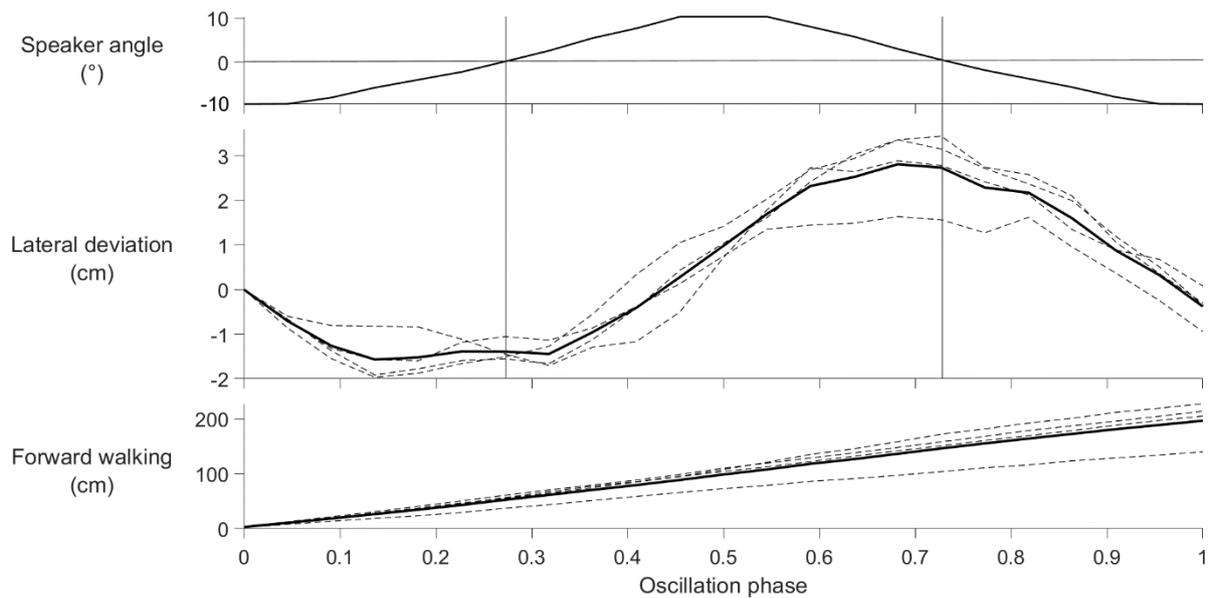


Fig. 4.9 Mean responses for one speaker oscillation from 10° right to 10° left. The dashed lines are responses of individual crickets and the thick straight lines show the overall mean ($n = 4$ crickets).

The crickets consistently walked forward (Fig. 4.9 bottom), and by the end of a speaker oscillation they had walked forward 197 ± 62 cm ($n=4$ crickets; mean \pm 95% C.I.). Their forward position depended on the phase of the speaker oscillation (repeated measures ANOVA, $F_{22,66}=49.584$, $P<0.0001$).

The mean values of the orientation angles of the crickets show that they generally walked towards the correct side, however they understeered compared to the ideal response (Fig. 4.10). overall, the position of the speaker had a significant effect on the orientation angle (repeated measures ANOVA, $F_{20,60}= 3.6078$, $P<0.001$, and, according to multiple comparisons tests, the angular resolution of the crickets for this paradigm was 7° to the right and 10° to the left (Tukey-Kramer test, $P<0.01$). However, this estimate of the angular resolution is conservative, because the small number of crickets ($n=4$) that responded to this speaker movement resulted in long confidence intervals (Fig. 4.10).

Tests with two static speakers

Out of 26 crickets tested, five steered both towards the speakers when these were positioned either at $\pm 5^\circ$ and or at $\pm 45^\circ$ (Fig. 4.11A). The lateral deviation towards the speakers at $\pm 5^\circ$ was generally

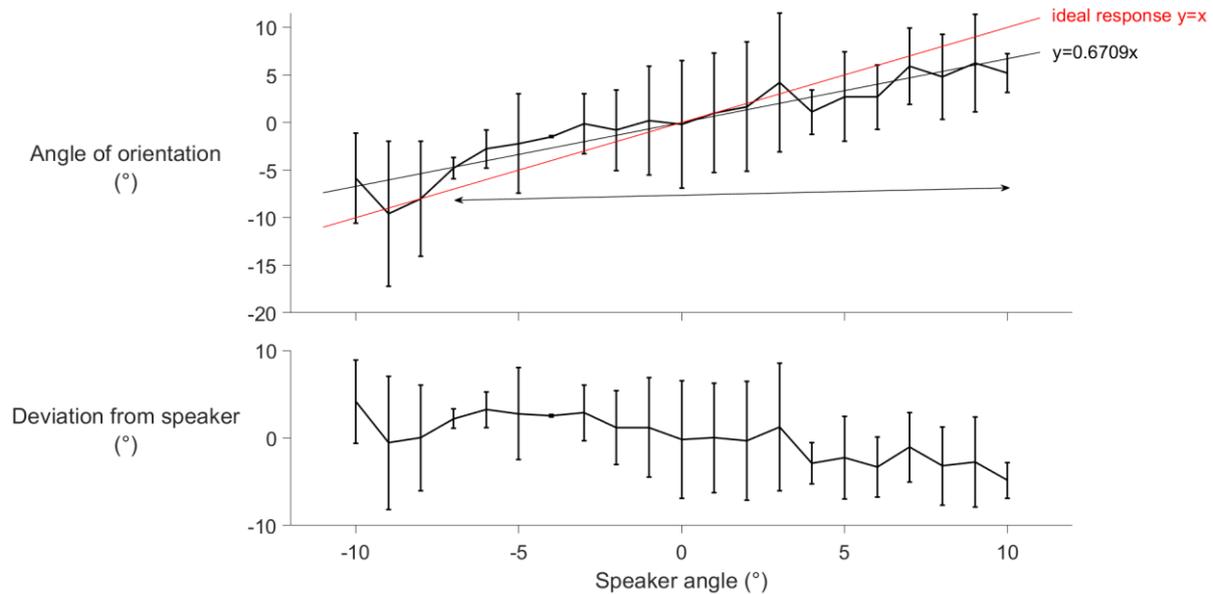


Fig. 4.10 Angle of orientation and deviation of crickets from the speaker angle for tests with the speaker moving from 10° right to 10° left (error bars indicate 95% C.I.; $n=4$ crickets). The speaker angle has a significant effect on the angle of orientation of the crickets (repeated measures ANOVA, $F_{20,60}=3.6078$, $P<0.001$). The double arrow shows the angular resolution of the crickets around the midline (Tukey-Kramer test, $P<0.01$).

“noisier” than towards the speakers at $\pm 45^\circ$, which could be indicative of phonotaxis towards 5° being more challenging, as the binaural cues are smaller. On average, the crickets changed their walking direction after a short delay – shorter than the duration of a chirp – when the presentation of the calling song switched from left to right and vice versa (Fig. 4.11B). The crickets steered towards individual chirps of the calling song, as indicated by the “ripples” in the lateral deviation that are coupled to the presentation of the chirps. This was more noticeable in the lateral deviation in response to the speakers at $\pm 45^\circ$.

On average, the crickets deviated less laterally when the speakers were set at $\pm 5^\circ$ than at $\pm 45^\circ$ (Fig. 4.12 middle). The maximum lateral deviation to the right was 1.9 ± 0.5 cm for the 5° paradigm and 4.8 ± 2.8 cm for the 45° paradigm. The corresponding values for the maximum lateral deviation to the left were 2.7 ± 1.1 cm and 7.6 ± 4.3 cm. There was no significant interaction between the *side* (left or right speaker) and *angle* factors (two-way ANOVA, $F_{1,16}=1.063$, $P=0.318$). Due to the left-right symmetry of the acoustic stimulus, there was no significant effect of the *side* factor on the lateral

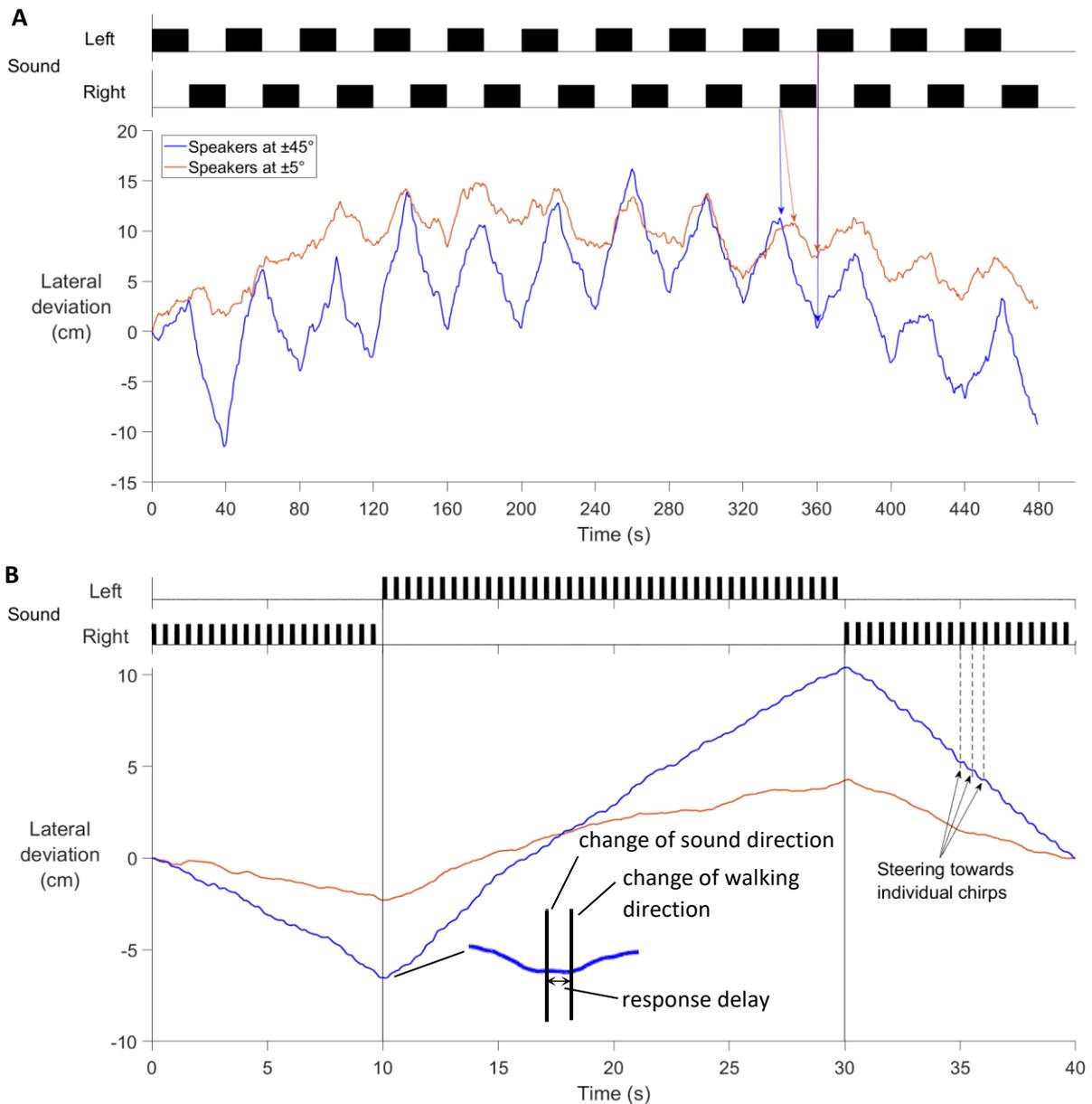


Fig. 4.11 Tests with speakers positioned at $\pm 5^\circ$ and $\pm 45^\circ$. **(A)** Response of a cricket. The arrows point to events when the cricket switches side of walking in response to the presentation of the calling song switching side. **(B)** Average response of the cricket for one period of the acoustic stimulus. The ripples in the lateral deviation indicate steering towards individual chirps of the calling song.

deviation (two-way ANOVA, $F_{1,16}=3.665$, $P=0.074$). There was however a significant effect of the *angle* factor on the lateral deviation (two-way ANOVA, $F_{1,16}=16.551$, $P<0.001$).

The crickets walked consistently forward (Fig. 4.12 bottom), and by the end of one sequence of the acoustic stimulus, which lasted 40 s, the crickets had walked forward 164.8 ± 52.2 cm ($n=5$

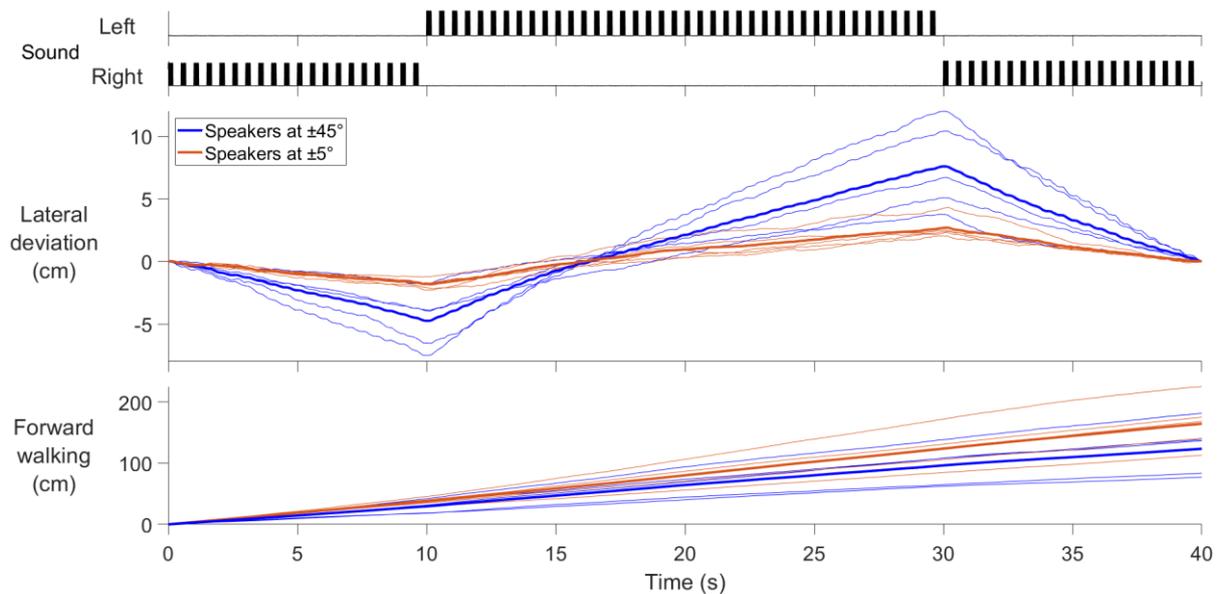


Fig. 4.12 Mean responses for one period of the acoustic stimulus. The thin lines show responses of individual crickets, and the thick lines show the average ($n=5$ crickets).

crickets; mean \pm 95% C.I.) towards the speakers at $\pm 5^\circ$ and 123.8 ± 53.9 cm towards the speakers at $\pm 45^\circ$. The difference between these two forward distances is significant (paired-sample t -test, $p < 0.001$).

The response delays for the right-to-left sound switch are 202 ± 85 ms for the 5° paradigm and 136 ± 55 ms for the 45° paradigm. The corresponding response delays for the left-to-right switch are 164 ± 83 ms and 160 ± 62 ms. Again, there is no significant interaction between the *side* (right-to-left or left-to-right sound switch) and *angle* factors (two-way ANOVA, $F_{1,16} = 1.410$, $P = 0.252$). There is also no significant effect of the *side* and *angle* factors on the response delay (two-way ANOVA; *side*: $F_{1,16} = 0.072$, $P = 0.792$; *angle*: $F_{1,16} = 1.798$, $P = 0.199$).

The crickets also steered towards individual chirps when the calling song was presented continuously from one side (Fig. 4.13). The response delays to chirps coming from the left are 117 ± 65 ms and 86 ± 14 ms for the 5° and 45° paradigms respectively. The corresponding response delays for chirps from the right are 104 ± 46 ms and 75 ± 6 ms. There is no significant interaction between the *side* and *angle* factors and no significant effect of the *side* and *angle* on the response delay (two-way ANOVA, *side-angle* interaction: $F_{1,16} = 0.009$, $P = 0.925$; *angle*: $F_{1,16} = 4.127$, $P = 0.059$; *side*: $F_{1,16} = 0.669$, $P = 0.425$).

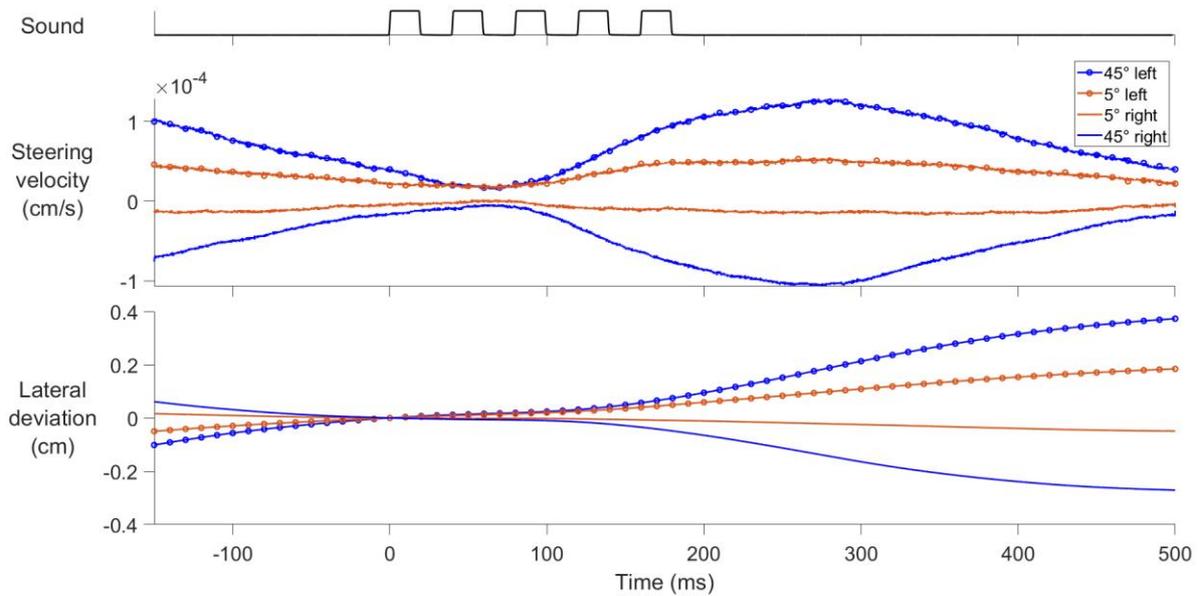


Fig. 4.13 Mean velocities and lateral deviations over one chirp of the calling song ($n = 5$ crickets, $N = 480$ chirps per paradigm per cricket).

Discussion

In this study I tested how accurately crickets walking on a trackball can orient towards a constantly moving sound source and how they respond to changes in direction of stationary stimuli. For a functional analysis, a group of crickets was tested after fixing their prothorax against the mesothorax.

Phonotaxis towards a moving sound source

I conducted two sets of experiments, with the speaker oscillating either between 45° right and 45° left or between 10° right and 10° left. In both sets the lateral deviations of the crickets showed that they were able to follow the speaker movement (Fig. 4.3 and 4.7). The fact that the crickets were able to follow the speaker movement from 10° right to 10° left (Fig. 4.7 and 4.9) means that their angular resolution in their frontal range is at least 10° for this dynamic situation. Based on statistical analysis using multiple comparisons, the angular resolution of the crickets in the frontal range was 11° to the right and 6° to the left for the speaker movement at $\pm 45^\circ$ (Fig. 4.6), and 7° to the right and 10° to the left for the speaker movement at $\pm 10^\circ$ (Fig. 4.10). The left-right asymmetry in the angular resolution could be the result of fluctuations of the sound intensity on top of the trackball in the range of ± 0.75 dB depending on the position of the speaker. These fluctuations occurred despite the symmetry of the anechoic chamber and the use of anti-reflective foam. Measurements of the tympanal vibrations

of crickets using laser vibrometry showed that the interaural intensity differences increase with a slope of 0.4 dB/° for angles 0-30° (Schöneich and Hedwig, 2010), therefore the fluctuations of the sound intensity may have had an effect on the responses of the crickets.

All previous studies on the sound localization ability of insects were conducted using static sound sources, and the results depended not only on the species studied but, in the case of crickets, also the method used (see Chapter 1 for review). In the study most akin to mine, Schöneich and Hedwig (2010) used the same trackball system but a static sound source and reported an angular resolution of 1° when crickets were exposed to the same stimulus pattern over 30 s. The poorer localization ability reported here is likely because orientation towards a moving sound source is more challenging due to the constantly changing binaural cues.

Localization of moving sound sources has been studied in humans (Harris and Sergeant, 1971; Perrott and Musicant, 1977; Getzmann and Lewald, 2007) and some animals, such as aerial predators that hunt using acoustic cues (owls: Wagner and Takahashi 1990, 1992; bats: Harem, Kleiser and Schuller, 1995; Wilson and O'Neill, 1998). The angular resolution of humans for static sound sources is 1-2° (Mills, 1958), but this number is, depending on the sound frequency, two to six times larger for slowly moving (2.8°/s) sound sources (Harris, 1972), and increases even more for higher sound source velocities (Perrott and Musicant, 1977). Humans tend to misperceive the position of a moving sound source in the direction of movement, a phenomenon termed *auditory representational momentum*, and mental extrapolation of past trajectory information is a possible explanation for it (Getzmann and Lewald, 2007). In my tests, most crickets crossed from left to right and vice versa before the speaker, as indicated by the reversal points of the lateral deviations that precede the zero crossings of the speaker (Fig. 4.5 and 4.9). It is not clear whether this phenomenon is a byproduct of the crickets tracking the speaker as the directional cues become smaller towards the midline or they can “anticipate” the speaker position based on the direction of its movement. Crickets have excellent capabilities for olfactory and visual learning (review: Mizunami and Matsumoto, 2017), however, learning and memory of acoustic stimuli has not been investigated and reported.

Several studies in mammals (Yin and Kuwada, 1983; Rauschecker and Harris, 1989; Harem, Kleiser and Schuller, 1995; Spitzer and Semple, 2000) and the barn owl (Wagner and Takahashi, 1990, 1992) demonstrated the existence of motion direction- as well as velocity-sensitive neurons in the ascending auditory system, but it has not been tested if auditory neurons of insects possess such properties. In general, male crickets remain stationary when they sing, therefore the ability to localize a moving sound source would be of limited use in the context of mating. However, awareness of the direction and velocity of an echolocating bat could be useful in evading it (Moiseff, Pollack and Hoy, 1978). Ascending Neuron 2 (AN2) is sensitive to ultrasound and triggers evasive behavior (Moiseff and Hoy, 1983); it therefore makes a good candidate to test for encoding the direction of movement and/or the velocity of ultrasonic sound sources.

Effect of fixing the prothorax on auditory steering

The results from Chapters 2 and 3 showed that during phonotaxis the prothorax makes two types of lateral movement: (i) a rhythmic left-right movement coupled to the stepping cycle and (ii) an auditory-induced movement towards the sound source. When the speaker is positioned at 0°, no auditory-induced bending of the prothorax is expected in optimally performing crickets, since they would not steer, but walk straight ahead instead. As the speaker moves laterally, the auditory-induced bending of the prothorax is expected to increase, therefore the effect of fixing its position should become more obvious. Even though fixing the prothorax against the mesothorax did not have a statistically significant effect on any of the parameters measured during auditory steering (Fig. 4.4-4.6; Table 4.1), the crickets with the fixed prothorax generally understeered, which was more evident during steering towards the more lateral stimulus angles (Fig. 4.6). Insects exhibit a resilient and flexible ability to retain their ability for locomotion even after substantial changes to their body properties, such as leg amputation (Hughes, 1957; Graham, 1977; Grabowska *et al.*, 2012; Owaki *et al.*, 2021). Because the angle of the prothorax relative to the median line remains small (approximately 1.5° for straight ahead walking and 5° for orientation towards a sound source at 45°; see Chapter 2),

it is possible that the crickets with the fixed prothorax can compensate for the lack of prothoracic mobility, e.g. by adjusting the trajectories of the front legs.

The contribution of the prothoracic movements in the locomotion and steering of insects has generally not been explored, and to my knowledge only the praying mantis, which has a movable joint between the prothorax and the pterothorax, has been shown to bend the prothorax to orient towards a prey (Yamawaki *et al.*, 2011) or to steer during swimming (Miller, 1972). Still, fixing the prothorax of mantises with wax – like I did for crickets – did not have an effect on their tracking and striking of targets (Prete *et al.*, 2012).

Tests with two static speakers

The crickets were more accurate when they steered towards the static speakers than towards the moving speaker, which shows that orientation towards a moving sound source is more challenging and provides less reliable directional cues. The individuals that responded to the tests with the two speakers steered consistently to the correct side both when the speakers were positioned at $\pm 5^\circ$ and when they were positioned $\pm 45^\circ$ (Fig. 4.11 and 4.12). This means that for static sound sources the angular resolution of the crickets was at least 5° , and the notion that the crickets face an area of uncertainty within $\pm 25^\circ$ in their frontal range, as suggested by Y-maze tests, (Rheinlaender and Blätgen, 1982) should be reconsidered. Unlike with the trackball tests, the structure of the Y-maze may cause acoustic echoes that bias the cricket to steer towards the “wrong” path of the junction. In addition, in a Y-maze the angle of the cricket’s longitudinal axis relative to the sound source cannot be controlled as reliably as on a trackball.

The crickets changed their walking direction in response to the presentation of the calling song switching from left to right and vice versa with a delay of 164-202 ms when the speakers were positioned at $\pm 5^\circ$ and 136-160 ms when they were positioned at $\pm 45^\circ$. The effect of the angle of the speakers on the response delay was not statistically significant, but this could be due to the small sample size ($n=5$). The crickets also steered towards individual chirps when the calling song was presented from one side (Fig. 4.13). The response delays towards individual chirps were 104-117 ms

when the speakers were positioned at $\pm 5^\circ$ and 75-86 ms when they were positioned at $\pm 45^\circ$. Again, there was no significant effect of the angle of the speakers on the response delay.

Phonotaxis towards the speakers at $\pm 5^\circ$ was generally “noisier”, and the reversal points of the lateral deviation were not as “sharp” as the reversal points when steering towards $\pm 45^\circ$ (Fig. 4.11). This suggests that orientation towards 5° was more challenging, possibly because the binaural cues around the median line are small (Schöneich and Hedwig, 2010). For a stimulus angle of 5° the sound intensity differs only by 0.2 dB between the two sides of the cricket (Michelsen, Popov and Lewis, 1994). Measurements of the tympanic membrane oscillations for stimulus angles from 0° to 30° showed that the interaural amplitude difference increase with a slope of 0.4 dB/ $^\circ$ (Schöneich and Hedwig, 2010). According to this linear model, the interaural amplitude difference at 5° is 2 dB and, assuming the model can be applied up to 45° , 18 dB at 45° . At the neuronal level, for a stimulus angle around 5° , the difference between the action potentials of the ipsilateral and contralateral AN1 is 1-3 action potentials per chirp and the difference in the response latency is less than 0.25 ms (Lv, Zhang and Hedwig, 2020). At 45° the corresponding differences in AN1 activity are up to 16 action potentials per chirp and around 1 ms. If auditory input to the tibial motoneurons of the front legs and the motoneurons of pronotal muscle 56 is indeed via a descending pathway (Baden and Hedwig, 2008; see Chapter 3), measuring the spike and latency differences of descending auditory neurons (Staudacher, 2001; Zorović and Hedwig, 2011) is probably more relevant with regards to the directionality of auditory steering. At the level of the descending auditory pathway the binaural differences are expected to be equal to or lower than at the level of AN1, unless there is a mechanism that enhances the binaural cues in the brain or in the descending pathway. A mechanism for the enhancement of binaural cues has been described in the prothoracic ganglion, where omega neuron 1 (ON1) enhances the binaural cues in the activity forwarded to the ascending neurons via reciprocal inhibition (Wohlers and Huber, 1982; Boyd and Lewis, 1983; Wiese and Eilts-Grimm, 1985; Larsen, Kleindienst and Michelsen, 1989).

Chapter 5: General discussion

Main findings

This thesis investigated the motor responses and the accuracy of female crickets (*Gryllus bimaculatus*) as they perform phonotaxis towards the male calling song. In Chapter 2, high-speed video recordings showed that when initially motionless crickets commence phonotaxis, their appendages and body parts are activated from anterior to posterior, with the antennae moving first, approximately 180 ms after the start of a chirp, and the rest follow in this order: head, prothorax, front legs, and middle legs. During phonotaxis, the antennae move continuously side-to-side in a rhythmic pattern and at the same time exhibit an auditory-induced movement towards the speaker playing the calling song. Furthermore, the prothorax moves continuously side-to-side in accordance with the stepping cycle and superimposed on this rhythmic movement is an auditory-induced tilt towards the speaker playing the calling song. In line with what has been described before (Witney and Hedwig, 2011), the trajectories of the tarsi of the front and middle legs tilted towards the side of the speaker playing the calling song.

In Chapter 3, I explored the function of the antennal muscles of the scape and of several thoracic muscles for potential contribution to the antennal and prothoracic movements during phonotaxis, respectively. The activity of antennal adductor muscle 6 was coupled to the adduction of the antenna during the contralateral presentation of the calling song, while the activity of abductor muscle 7 was coupled to the abduction of the antenna during the ipsilateral presentation of the calling song. Of all the thoracic muscles tested, only the activity of pronotal muscle 56 was coupled to the movement of the prothorax during phonotaxis, that is both the auditory-induced movement and the rhythmic movement coupled to the stepping cycle.

In Chapter 4, I tested how accurately crickets can orient towards a moving and towards a static sound source. The moving speaker paradigm was applied for the first time to cricket phonotaxis. The angular resolution in the frontal range of the crickets was 6°-11° during phonotaxis towards a speaker

moving constantly at a velocity of $1.1^\circ/\text{s}$. When the calling song was presented from two static speakers, the crickets were able to steer correctly to the side of the active speaker both when the speakers were positioned 45° left and 45° right and when they were positioned 5° left and 5° right. Therefore, the angular resolution in the frontal range was at least 5° for static sound sources. The angular resolution is lower for orientation towards a moving sound source likely because tracking its motion is challenging, since the binaural cues change constantly and need to be updated during their integration into the auditory-induced steering responses.

Integration of sensory input into motor responses

Phonotaxis requires two types of motor activity: locomotion and auditory-induced steering. Locomotion, i.e. walking, requires the coordinated contractions of many muscles, whose motoneurons have been shown to be driven by the contribution of specialized neural networks called central pattern generators (CPGs) that can intrinsically generate a rhythmic motor output (review: Marder and Rehm, 2005). The CPGs that control leg movements and generate coordinated walking reside in the nerve cord, but descending commands from the brain are instrumental for activating and manipulating them to achieve goal directed locomotion (review: Bidaye, Bockemühl and Büschges, 2018). The study of walking CPGs in insects has focused on how the joints of a single leg and the interleg joints are coordinated during walking (Bidaye, Bockemühl and Büschges, 2018), however, as shown in Chapters 2 and 3 the prothorax also moves rhythmically side-to-side in accordance to the stepping cycle, and such rhythmic movement is likely generated by a CPG as well. Moreover, during phonotaxis the antennae move together side-to-side in a rhythmic pattern, also likely generated by a CPG.

The other motor activity component of phonotaxis, auditory-induced steering, requires the nervous system to extract the behaviorally relevant features from the auditory input and then transform and integrate them into a steering motor response. Behavioral studies can identify the behaviorally relevant features of the calling song, which can then guide the use of appropriate stimuli to probe the neural circuit that is assumed to extract such features. An example of this approach was

the use of behavioral tests to analyze the selectivity of female *G. bimaculatus* with respect to temporal features of the calling song (Kostarakos and Hedwig, 2012), followed by physiological studies that identified a brain circuit for the detection of the pulse pattern of the calling song via a delay-line and coincidence detector mechanism (Schöneich, Kostarakos and Hedwig, 2015).

The high-speed video recordings in Chapter 2 showed that when motionless crickets are presented with the calling song, they commence phonotaxis by activating their body parts and appendages from anterior to posterior, with the antennae being the first appendages to move approximately 180 ms after the start of a chirp. The anterior to posterior order of activation must be achieved via a pathway descending from the brain. Around 200 interneurons descend from the brain (Staudacher, 1998), some of which exhibit auditory responses (Staudacher and Schildberger, 1998; Staudacher, 2001; Zorović and Hedwig, 2011), however none has been shown to provide input to motoneurons yet. When motionless crickets are presented with the calling song, the auditory input first needs to ascend to the brain so that the calling song is recognized, and since the antennal motoneurons are also located in the brain, specifically the deutocerebrum (Honegger *et al.*, 1990), they are the first to receive auditory input. The two identified ascending auditory interneurons, AN1 and AN2, project from the prothoracic ganglion into the protocerebrum (Moiseff and Hoy, 1983; Schildberger, 1984) and have no collaterals in the suboesophageal ganglion, therefore auditory input to the antennal motoneurons in the deutocerebrum must be indirect, via brain interneurons that transfer the auditory signal from the protocerebrum.

Pattern recognition controls auditory steering transiently (Poulet and Hedwig, 2005), therefore in crickets that have established pattern recognition and are performing phonotaxis, it is possible that the auditory input does not have to ascend to the brain for a motor command to descend but rather is integrated at the thoracic level to the walking motor activity instead. This means that the pathway through which the auditory input is integrated into the steering responses could depend on the behavioral state of the crickets, i.e. whether they are transitioning from motionlessness to the

initiation of phonotaxis or continuously performing phonotaxis. The behavioral state-dependence of sensorimotor integration has been shown in other systems, such as the fly's gaze-stabilization system, which visually detects self-rotations and generates compensatory head movements to maintain a level gaze during flight (Hengstenberg, 1991). The activity of the ventral cervical nerve motoneuron cell, which controls head movements of the fly, is gated by haltere movements: unless visual motion is combined with flight activity or wind stimuli, the spike activity is not modulated in a directionally selective way (Haag, Wertz and Borst, 2010). Accordingly, in crickets the integration of the auditory input at the level of the motor activity – instead of integration via a descending pathway – could be gated by pattern recognition and locomotion.

As shown in Chapters 2 and 3, crickets performing phonotaxis tilt rapidly the antennae and the prothorax towards the active speaker, even when the presentation of the calling song alternates between a left and right speaker every second chirp or every chirp. The auditory-induced movements of the antennae and the prothorax are superimposed on the rhythmic components of their movements. The muscles of the scape that control the antennal movements in the horizontal plane are innervated by a total of 7 motoneurons that are located in the deutocerebrum (Honegger *et al.*, 1990). Some antennal motoneurons likely receive input from a CPG that generates the rhythmic pattern of the antennal movements, while some receive an indirect auditory input via brain interneurons. Accordingly, pronotal muscle 56, whose activity is coupled both to the rhythmic and auditory-induced prothoracic movements, is innervated by 3 motoneurons that project to the dorsal side of the prothoracic ganglion (Honegger *et al.*, 1984), and their neurites do not overlap with the auditory neuropil in the ventral side (Esch, Huber and Wohlers, 1980; Wohlers and Huber, 1985). One or more motoneurons of muscle 56 likely receive input from the walking CPG that generates the rhythmic prothoracic movement that is coupled to the stepping cycle, and one or more motoneurons receive indirect auditory input. To answer whether during phonotaxis the auditory input to the motoneurons of muscle 56 is via local prothoracic interneurons or via a descending pathway, it is crucial to determine whether the response delay of muscle 56 to the acoustic stimulus is shorter or

longer than the response delay of the antennal muscles. Shorter delay would mean that muscle 56 receives auditory input before the antennal muscles, therefore the auditory input would have to be via prothoracic interneurons. In contrast, longer delay would point towards auditory input via a descending pathway. During phonotaxis the response delay of the antennal muscles to the calling song was 90-180 ms, while the response delay of muscle 56 was only 37-75 ms, indicating a prothoracic pathway. However, the antennal muscles and muscle 56 were recorded in different animals, and the sample size for the recordings of the antennal muscles was small ($n=2$ crickets). Moreover, a response delay of 37-75 ms is likely too long for auditory-to-motor integration at the level of the prothoracic ganglion and points towards a descending command.

The continuous rhythmic movements of the antennae and the prothorax coupled to the walking activity make the identification of the auditory-induced responses challenging, which is why I opted to examine their temporal activation pattern at the commencement of phonotaxis in initially motionless crickets. However, as explained above, the auditory-to-motor integration pathway when crickets commence phonotaxis may differ from the pathway during phonotaxis. It may be possible to examine the temporal relationship of the auditory-induced movements of the antennae and the prothorax during phonotaxis by averaging a large sample, so that the continuous rhythmic movements are nulled and only the auditory-induced movements remain. Still, as shown in Chapter 3 the antennae can be abducted ipsilaterally before the contraction of abductor muscle 7, most likely due to passive elastic properties of the muscles of the scape and the scape-pedicle joint. Therefore, the most reliable way to determine if the motoneurons of pronotal muscle 56 receive auditory input before the motoneurons of antennal abductor muscle 7 would be to record simultaneously the activities of muscles 7 and 56 in crickets performing phonotaxis.

The role of the antennae during phonotaxis

The antennae are mobile multimodal sensory organs that monitor the frontal surroundings of the insect (Allgäuer and Honegger, 1993). They are the primary olfactory organs (Keil, 1992; Hallem, Ho and Carlson, 2004) but respond to tactile stimuli too (Dürr *et al.*, 2001; Okada and Toh, 2004, 2006;

Krause and Dürr, 2012; Mongeau *et al.*, 2013). Antennal movements and postures play an integral role in many behaviors, such as fighting (Alexander, 1961; Sakura and Aonuma, 2013; Aonuma, 2017), courtship (Loher and Rence, 1978), flight (Gewecke and Heinzel, 1980), and investigating objects (Okada and Toh, 2006; Harley, English and Ritzmann, 2009).

Tracking of the antennal movements during phonotaxis in Chapters 2 and 3 showed that crickets continuously move their antennae side-to-side, a behavior that has also been observed in other insects during walking (e.g. cockroaches: Okada and Toh, 2004; Harley, English and Ritzmann, 2009; stick insects: Dürr *et al.*, 2001; Krause and Dürr, 2012; beetles: Pelletier and McLeod, 1994; Zurek and Gilbert, 2014) and serves to explore the frontal surroundings. In some insects, the antennae are of similar length or longer than the legs; as a result, the moving antennae can touch objects within reach of the front legs and allow the insect to adjust its body axis (Pelletier and McLeod, 1994), walking height (Harley, English and Ritzmann, 2009), and/or the execution of aimed limb movements, such as reach-to-grasp movements (Schütz and Dürr, 2011; Dürr *et al.*, 2018).

When walking stick insects steer in response to a visual stimulus, the optomotor response of the antennae leads that of the locomotion (Dürr and Ebeling, 2005). Similarly, the high-speed videos of Chapter 2 showed that when crickets commence phonotaxis, they move the antennae towards the sound source 10-30 ms before they move the legs, which possibly allows them to adjust their locomotion based on antennal sensory input.

Trackball tests showed that if a cricket performing phonotaxis touches an object with its antennae, it ceases walking to explore the object (Haber Kern and Hedwig, 2016). Similar behavior for obstacle negotiation has been reported in beetles (Pelletier and McLeod, 1994), cockroaches (Harley, English and Ritzmann, 2009), and stick insects (Schütz and Dürr, 2011). Tactile sensing of an obstacle may allow the insect to determine the appropriate response to overcome the obstacle, such as climbing, tunneling, jumping, escaping, or turning.

Sound localization

I tested the accuracy of crickets performing phonotaxis towards static and moving sound sources. When the presentation of the calling song alternated between two static speakers positioned 5° left and 5° right, some individuals steered consistently correctly towards the active speaker. This means that for a static sound source, the angular resolution in the frontal range was at least 5°. At a 5° sound incidence angle the difference in the sound intensity between the left and right side of the cricket is around 0.2 dB (Michelsen, Popov and Lewis, 1994); however, the binaural difference of the amplitudes of the tympanal oscillations is around 2 dB (Schöneich and Hedwig, 2010). At the level of the auditory afferents, the binaural difference of the response latencies increases with a slope of 42 $\mu\text{s}/^\circ$ (Schöneich and Hedwig, 2010), which corresponds to 210 μs at 5°. Many auditory systems exploit smaller binaural differences in response latency for directional hearing (Carr and Macleod, 2010). For example, the parasitoid fly *Ormia ochracea* exploits a difference in response latencies that increases with a slope of 3.5 $\mu\text{s}/^\circ$ from 30° contralaterally to 30° ipsilaterally to achieve an angular resolution of 2° (Mason, Oshinsky and Hoy, 2001). At the level of ascending neuron AN1 of crickets, the binaural difference in action potentials at a sound incidence angle of 5° is 1-3 action potentials/chirp and the binaural difference of the response latencies is less than 250 μs (Lv, Zhang and Hedwig, 2020). As discussed above, the auditory responses are possibly induced via a descending pathway, in which case the bilateral differences in the activity of the descending pathway are more relevant to auditory steering than the binaural cues at the level of the afferents or the ascending pathway. Specifically, descending auditory neurons that respond with a latency of 25-47 ms at the level of the connectives between the suboesophageal and prothoracic ganglia (Staudacher, 2001) are candidates for integrating directional cues into a command to drive motor responses at the level of the prothoracic ganglion.

Due to the small binaural differences at small sound incidence angles, there was a long-standing assumption that crickets face an area of ambiguity with a width of 50° and cannot steer correctly to the side of the sound source when the calling song is presented from within this area. This hypothesis was supported by Y-maze tests, where crickets could not consistently turn correctly if the

sound was presented from within 25° off their length axis (Rheinlaender and Blätgen, 1982). Furthermore, crickets walking on a locomotion compensator oscillated 30-60° off the sound direction (Schmitz, Scharstein and Wendler, 1982), suggesting low phonotactic accuracy. However, this and a previous study that showed that crickets can resolve sound incidence angles that deviated just 1° from their length axis (Schöneich and Hedwig, 2010) demonstrate that crickets can be significantly more accurate than previously believed. A possible reason for this apparent discrepancy is that the older studies described the average sound localization capabilities of a population, whereas this study and the study by Schöneich and Hedwig (2010) aimed to describe the resolution that the cricket auditory is capable of by analyzing the performance of the most acute individuals. Moreover, older trackball systems compensated the crickets' walking movements electro-mechanically (Wendler *et al.*, 1980; Schmitz, Scharstein and Wendler, 1982), which may have had an effect on the walking paths, due to the delay between the cricket movement and the compensatory rotation of the trackball (Weber, Thorson and Huber, 1981).

When the cricket performed phonotaxis towards a slowly moving (1.1°/s) sound source, their angular resolution was 6-11°. The reason why orientation towards a moving sound source was less accurate than towards a static one is likely because the binaural cues from a moving sound source change constantly, making localization more challenging. Several mammals (Yin and Kuwada, 1983; Rauschecker and Harris, 1989; Harem, Kleiser and Schuller, 1995; Spitzer and Semple, 2000) and the barn owl (Wagner and Takahashi, 1990, 1992) possess motion direction- as well as velocity-sensitive neurons in the ascending auditory system, however, to my knowledge the responses of insect auditory neurons to moving sound sources have not been tested yet. In crickets, auditory neurons that detect the motion direction of a sound source could facilitate bat evasion, therefore ascending neuron AN2, which is sensitive to ultrasound (Moiseff and Hoy, 1983; Fullard, Ratcliffe and Guignon, 2005), would be a candidate to test for such properties.

Some crickets switched walking from left to right and vice versa before the moving speaker crossed the midline, however it is not clear whether they did so because they “anticipated” the position of the speaker or because they could not localize accurately due to the small binaural cues around the midline. Extrapolation of the position of a moving sound source based on its previous positions implies memory and ability to learn. The ability to learn has been demonstrated for many insect species, including honeybees (Bitterman *et al.*, 1983; Gerber *et al.*, 1998; Gaurav, 2007; Giurfa and Sandoz, 2012), fruit flies (Tully and Quinn, 1985), crickets (Matsumoto and Mizunami, 2000), cockroaches (Balderrama, 1980), ants (Dupuy *et al.*, 2006), and moths (Daly and Smith, 2000). *G. bimaculatus* has a strong capability for visual and olfactory learning and memory (review: Mizunami and Matsumoto, 2017), however auditory learning and memory has not been investigated and reported.

Conclusions

In this thesis, I have analyzed the auditory-to-motor responses and directional accuracy of cricket phonotaxis. The antennae and the prothorax exhibit auditory-induced responses that are controlled by the antennal muscles of the scape and by pronotal muscle 56, respectively. Based on the response latency of muscle 56 to the calling song and the location of its motoneurons, I suggested that it receives indirect auditory input via an unidentified descending pathway. Still, it is possible that the auditory-to-motor integration occurs at the level of the prothoracic ganglion. To resolve this query, I have recommended to record simultaneously the activities of antennal abductor muscle 7 and pronotal muscle 56 and compare their response latencies to the calling song. Shorter response latency of muscle 56 would point to auditory-to-motor integration at the level of the prothoracic ganglion, whereas longer response latency would point to integration via a descending pathway.

The angular resolution of the crickets was at least 5° for phonotaxis towards a static sound source and 6-11° for phonotaxis towards a slowly moving (1.1°/s) sound source. Phonotaxis towards a moving sound source was less accurate because the binaural cues change constantly, which makes

localization more challenging. Some animals possess neurons that respond to the motion direction and velocity of a sound source, and I recommended testing AN2 for such properties.

Bibliography

- Ache, J. M. and Matheson, T. (2012) 'Passive resting state and history of antagonist muscle activity shape active extensions in an insect limb', *Journal of Neurophysiology*, 107(10), pp. 2756–2768. doi: 10.1152/jn.01072.2011.
- Alexander, R. D. (1961) 'Aggressiveness, territoriality, and sexual behavior in field crickets (Orthoptera: Gryllidae)', *Behaviour*, 17(2–3), pp. 130–223. doi: 10.1163/156853961X00042.
- Alexander, R. D. (1962) 'Evolutionary change in cricket acoustical communication', *Evolution*, 16(4), p. 443. doi: 10.2307/2406178.
- Allgäuer, C. and Honegger, H. W. (1993) 'The antennal motor system of crickets: modulation of muscle contractions by a common inhibitor, DUM neurons, and proctolin', *Journal of Comparative Physiology A*, 173(4), pp. 485–494. doi: 10.1007/BF00193521.
- Angioy, A. M. *et al.* (2003) 'Extreme sensitivity in an olfactory system', *Chemical Senses*, 28(4), pp. 279–284. doi: 10.1093/chemse/28.4.279.
- Aonuma, H. (2017) 'Fighting behavior: Understanding the mechanisms of group-size-dependent aggression', in Horch, H. W. *et al.* (eds) *The Cricket as a Model Organism*. Tokyo: Springer Japan, pp. 197–210.
- Atkins, G. and Pollack, G. S. (1987) 'Correlations between structure, topographic arrangement, and spectral sensitivity of sound-sensitive interneurons in crickets', *Journal of Comparative Physiology*, 266, pp. 398–412.
- Avitabile, D. *et al.* (2009) 'Mathematical modelling of the active hearing process in mosquitoes', *Journal of the Royal Society Interface*, 7(42), pp. 105–122. doi: 10.1098/rsif.2009.0091.
- Baden, T. and Hedwig, B. (2008) 'Front leg movements and tibial motoneurons underlying auditory steering in the cricket (*Gryllus bimaculatus* deGeer)', *The Journal of Experimental Biology*, 211, pp. 2123–2133. doi: 10.1242/jeb.019125.
- Bailey, W. J. (1991) *Acoustic behaviour of insects: an evolutionary perspective*. London: Chapman and Hall.

- Bailey, W. J. and Field, G. (2000) 'Acoustic satellite behaviour in the Australian bushcricket *Elephantodeta nobilis* (Phaneropterinae, Tettigoniidae, Orthoptera)', *Animal Behaviour*, 59(2), pp. 361–369. doi: 10.1006/anbe.1999.1325.
- Bailey, W. J. and Thomson, P. (1977) 'Acoustic Orientation in the Cricket *Teleogryllus Oceanicus* (Le Guillou)', *Journal of Experimental Biology*, 67(1), pp. 61–75. doi: 10.1242/jeb.67.1.61.
- Balderrama, N. (1980) 'One trial learning in the American cockroach, *Periplaneta americana*', *Journal of Insect Physiology*, 26(8), pp. 499–504. doi: 10.1016/0022-1910(80)90123-7.
- Bentsen, C. L. *et al.* (2006) 'Complex multivariate sexual selection on male acoustic signaling in a wild population of *Teleogryllus commodus*', *The American naturalist*, 167(4). doi: 10.1086/501376.
- Bidaye, S. S., Bockemühl, T. and Büschges, A. (2018) 'Six-legged walking in insects: How CPGs, peripheral feedback, and descending signals generate coordinated and adaptive motor rhythms', *Journal of Neurophysiology*, 119(2), pp. 459–475. doi: 10.1152/jn.00658.2017.
- Bitterman, M. E. *et al.* (1983) 'Classical conditioning of proboscis extension in honeybees (*Apis mellifera*)', *Journal of comparative psychology (Washington, D.C. : 1983)*, 97(2), pp. 107–119. doi: 10.1037/0735-7036.97.2.107.
- Böhm, H. and Schildberger, K. (1992) 'Brain neurones involved in the control of walking in the cricket *Gryllus Bimaculatus*', *Journal of Experimental Biology*, 166(1), pp. 113–130. doi: 10.1242/jeb.166.1.113.
- Boyan, G. S. (1993) 'Another look at insect audition: The tympanic receptors as an evolutionary specialization of the chordotonal system', *Journal of Insect Physiology*, 39(3), pp. 187–200. doi: 10.1016/0022-1910(93)90088-9.
- Boyd, P. and Lewis, B. (1983) 'Peripheral auditory directionality in the cricket (*Gryllus campestris* L., *Teleogryllus oceanicus* Le Guillou)', *Journal of Comparative Physiology A*, 153, pp. 523–532.
- Bregman, A. S. (1990) *Auditory scene analysis: The perceptual organization of sound*. Cambridge, MA: MIT Press.
- Brooks, R. *et al.* (2005) 'Experimental evidence for multivariate stabilizing sexual selection', *Evolution*,

59(4), pp. 871–880. doi: 10.1111/j.0014-3820.2005.tb01760.x.

Carr, C. E. and Macleod, K. M. (2010) ‘Microseconds matter’, *PLoS Biology*, 8(6), pp. 6–8. doi: 10.1371/journal.pbio.1000405.

Cator, L. J. *et al.* (2009) ‘Harmonic convergence in the love songs of the dengue vector mosquito’, *Science*, 323(5917), 1077-1079.

Celiker, E., Jonsson, T. and Montealegre-Z, F. (2020) ‘On the tympanic membrane impedance of the katydid *Copiphora gorgonensis* (Insecta: Orthoptera: Tettigoniidae)’, *The Journal of the Acoustical Society of America*, 148(4).

Chapman, R. F. (2013) *The Insects: Structure and Function*. 5th edn. Cambridge: Cambridge University Press.

Coro, F. and Kössl, M. (1998) ‘Distortion-product otoacoustic emissions from the tympanic organ in two noctuid moths’, *Journal of Comparative Physiology A*, 183(4), pp. 525–531. doi: 10.1007/s003590050278.

Daly, K. C. and Smith, B. H. (2000) ‘Associative olfactory learning in the moth *Manduca sexta*’, *Journal of Experimental Biology*, 203(13), pp. 2025–2038. doi: 10.1242/jeb.203.13.2025.

Dobler, S., Heller, K. G. and von Helversen, O. (1994) ‘Song pattern recognition and an auditory time window in the female bushcricket *Ancistrura nigrovittata* (Orthoptera: Phaneropteridae)’, *Journal of Comparative Physiology A*, 175(1), pp. 67–74. doi: 10.1007/BF00217437.

Doherty, J. A. (1985) ‘Trade-off phenomena in calling song recognition and phonotaxis in the cricket, *Gryllus bimaculatus* (Orthoptera, Gryllidae)’, *Journal of Comparative Physiology A*, 156(6), pp. 787–801. doi: 10.1007/BF00610831.

Dupuy, F. *et al.* (2006) ‘Individual olfactory learning in *Camponotus* ants’, *Animal Behaviour*, 72(5), pp. 1081–1091. doi: 10.1016/j.anbehav.2006.03.011.

Dürr, V. *et al.* (2001) ‘The stick insect antenna as a biological paragon for an actively moved tactile probe for obstacle detection’, *Proceedings of CLAWAR conference*, pp. 87–96. Available at: <http://pub.uni->

bielefeld.de/pub?func=drec&id=1681344%5Cnhttp://books.google.com/books?hl=en&lr=&id=OgFzO9HIRzOC&oi=fnd&pg=PA87&dq=The+Stick+Insect+Antenna+as+a+Biological+Paragon+for+an+Actively+Moved+Tactile+Probe+for+Obstacle+Detection&ots=WJf9M0Y6qN&si.

Dürr, V. *et al.* (2018) 'Motor flexibility in insects: adaptive coordination of limbs in locomotion and near-range exploration', *Behavioral Ecology and Sociobiology*, 72(1). doi: 10.1007/s00265-017-2412-3.

Dürr, V. and Ebeling, W. (2005) 'The behavioural transition from straight to curve walking: Kinetics of leg movement parameters and the initiation of turning', *Journal of Experimental Biology*, 208(12), pp. 2237–2252. doi: 10.1242/jeb.01637.

Dürr, V., König, Y. and Kittmann, R. (2001) 'The antennal motor system of the stick insect *Carausius morosus*: Anatomy and antennal movement pattern during walking', *Journal of Comparative Physiology A*, 187(2), pp. 131–144. doi: 10.1007/s003590100183.

Ehret, G. (1978) 'Stiffness gradient along the basilar membrane as a basis for spatial frequency analysis within the cochlea', *Journal of the Acoustical Society of America*, 64(6), pp. 1723–1726. doi: 10.1121/1.382153.

Ellis, P. E. and Brimacombe, L. C. (1980) 'The mating behaviour of the egyptian cotton leafworm moth, *Spodoptera littoralis* (Boisd.)', *Animal Behaviour*, 28(4), pp. 1239–1248. doi: 10.1016/S0003-3472(80)80112-6.

Erber, J. *et al.* (1997) 'Tactile motor learning in the antennal system of the honeybee (*Apis mellifera* L.)', *Journal of Comparative Physiology A*, 181(4), pp. 355–365. doi: 10.1007/s003590050121.

Erber, J. *et al.* (1998) 'Tactile learning in the honeybee', *Journal of Comparative Physiology A*, 183(6), pp. 737–744. doi: 10.1007/s003590050296.

Esch, H., Huber, F. and Wohlers, D. W. (1980) 'Primary auditory neurons in crickets: Physiology and central projections', *Journal of Comparative Physiology A*, 137(1), pp. 27–38. doi: 10.1007/BF00656914.

Ewing, A. W. (1989) *Arthropod bioacoustic: Neurobiology and behavior*. Ithaca: Comstock/Cornell.

- Faure, P. A. and Hoy, R. R. (2000a) 'Neuroethology of the Katydid T-Cell I. Tuning and responses to pure tones', *Journal of Experimental Biology*, 203, pp. 3225–3242. Available at: [papers3://publication/uuid/78576775-D9AF-4D77-AA5D-B86A29C590DE](https://pubs3://publication/uuid/78576775-D9AF-4D77-AA5D-B86A29C590DE).
- Faure, P. A. and Hoy, R. R. (2000b) 'Neuroethology of the katydid T-cell II. Responses to acoustic playback of conspecific and predatory signals', *Journal of Experimental Biology*, 203, pp. 3243–3254. doi: 10.1242/jeb.203.21.3225.
- Fay, R. R. (1988) *Hearing in Vertebrates: A Psychophysics Databook*. Winnetka, Illinois: Hill-Fay Associates.
- Fay, R. R. and Popper, A. N. (2000) 'Evolution of hearing in vertebrates: The inner ears and processing', *Hearing Research*, 149(1–2), pp. 1–10. doi: 10.1016/S0378-5955(00)00168-4.
- Fletcher, N. H. and Thwaites, S. (1979) 'Acoustical analysis of the auditory system of the cricket *Teleogryllus commodus* (Walker)', *Journal of the Acoustical Society of America*, 66(2), pp. 350–357. doi: 10.1121/1.383668.
- Fonseca, P. J. and Correia, T. (2007) 'Effects of temperature on tuning of the auditory pathway in the cicada *Tettigetta josei* (Hemiptera, Tibicinidae)', *Journal of Experimental Biology*, 210(10), pp. 1834–1845. doi: 10.1242/jeb.001495.
- Fonseca, P. J., Münch, D. and Hennig, R. M. (2000) 'How cicadas interpret acoustic signals', *Nature*, 405(6784), pp. 297–298. doi: 10.1038/35012696.
- Fotowat, H. *et al.* (2009) 'A novel neuronal pathway for visually guided escape in *Drosophila melanogaster*', *Journal of Neurophysiology*, 102(2), pp. 875–885. doi: 10.1152/jn.00073.2009.
- Fotowat, H., Harrison, R. R. and Gabbiani, F. (2011) 'Multiplexing of Motor Information in the Discharge of a Collision Detecting Neuron during Escape Behaviors', *Neuron*, 69(1), pp. 147–158. doi: 10.1016/j.neuron.2010.12.007.
- Fullard, J. H., Forrest, E. and Surlykke, a (1998) 'Intensity responses of the single auditory receptor of notodontid moths: A test of the peripheral interaction hypothesis in moth ears', *The Journal of experimental biology*, 201, pp. 3419–24.

- Fullard, J. H., Ratcliffe, J. M. and Guignion, C. (2005) 'Sensory ecology of predator-prey interactions: Responses of the AN2 interneuron in the field cricket, *Teleogryllus oceanicus* to the echolocation calls of sympatric bats', *Journal of Comparative Physiology A*, 191(7), pp. 605–618. doi: 10.1007/s00359-005-0610-3.
- Fullard, J. H. and Yack, J. E. (1993) 'Evolution of insect hearing', *Trends in Ecology & Evolution*, pp. 248–252.
- Furukawa, N., Tomioka, K. and Yamaguchi, T. (1983) 'Functional anatomy of the musculature and innervation of the neck and thorax in the cricket, *Gryllus bimaculatus*', *Zoological Magazine*, 92, pp. 371–385.
- Gerber, B. *et al.* (1998) 'Temporal determinants of olfactory long-term retention in honeybee classical conditioning: Nonmonotonous effects of the training trial interval', *Neurobiology of Learning and Memory*, 69(1), pp. 71–78. doi: 10.1006/nlme.1997.3801.
- Gerhardt, H. C. and Huber, F. (2001) *Acoustic Communication in Insects and Anurans*. Chicago: University of Chicago.
- Getzmann, S. and Lewald, J. (2007) 'Localization of moving sound', *Perception and Psychophysics*, 69(6), pp. 1022–1034. doi: 10.3758/BF03193940.
- Gewecke, M. and Heinzl, H. (1980) 'Aerodynamic and Mechanical Properties of the Antennae as Air-Current Sense Organs in *Locusta migratoria*', *Comparative and General Pharmacology*, 366, pp. 357–366.
- Gillam, E. H. *et al.* (2011) 'Virtual bats and real insects: effects of echolocation on pheromone-tracking behavior of male corn earworm moths, *Helicoverpa zea*', *The Southwestern Naturalist*, 56(1), pp. 103–107. doi: 10.1894/JC-37.1.
- Giurfa, M. (2007) 'Behavioral and neural analysis of associative learning in the honeybee: A taste from the magic well', *Journal of Comparative Physiology A*, 193(8), pp. 801–824. doi: 10.1007/s00359-007-0235-9.
- Giurfa, M. and Sandoz, J. C. (2012) 'Invertebrate learning and memory: Fifty years of olfactory

conditioning of the proboscis extension response in honeybees', *Learning and Memory*, 19(2), pp. 54–66. doi: 10.1101/lm.024711.111.

Gollisch, T. *et al.* (2002) 'Energy integration describes sound-intensity coding in an insect auditory system', *Journal of Neuroscience*, 22(23), pp. 10434–10448. doi: 10.1523/jneurosci.22-23-10434.2002.

Göpfert, M. C. and Robert, D. (2001) 'Active auditory mechanics in mosquitoes.', *Proceedings of the Royal Society B: Biological Sciences*, 268(1465), pp. 333–9. doi: 10.1098/rspb.2000.1376.

Grabowska, M. *et al.* (2012) 'Quadrupedal gaits in hexapod animals - inter-leg coordination in free-walking adult stick insects', *Journal of Experimental Biology*, 215(24), pp. 4255–4266. doi: 10.1242/jeb.073643.

Graham, D. (1977) 'The effect of amputation and leg restraint on the free walking coordination of the stick insect *Carausius morosus*', *Journal of Comparative Physiology A*, 116(1), pp. 91–116. doi: 10.1007/BF00605519.

Greenfield, M. D. (2016) 'Evolution of Acoustic Communication in Insects', in Pollack, G. S. *et al.* (eds) *Insect Hearing*. Switzerland: Springer International Publishing, pp. 17–48.

Gu, J. J. *et al.* (2012) 'Wing stridulation in a jurassic katydid (insecta, orthoptera) produced low-pitched musical calls to attract females', *Proceedings of the National Academy of Sciences of the United States of America*, 109(10), pp. 3868–3873. doi: 10.1073/pnas.1118372109.

Haag, J., Wertz, A. and Borst, A. (2010) 'Central gating of fly optomotor response', *Proceedings of the National Academy of Sciences of the United States of America*, 107(46), pp. 20104–20109. doi: 10.1073/pnas.1009381107.

Haberkern, H. and Hedwig, B. (2016) 'Behavioural integration of auditory and antennal stimulation during phonotaxis in the field cricket *Gryllus bimaculatus*', *Journal of Experimental Biology*, 219(22), pp. 3575–3586. doi: 10.1242/jeb.141606.

Hallem, E. A., Ho, M. G. and Carlson, J. R. (2004) 'The molecular basis of odor coding in the *Drosophila* antenna', *Cell*, 117(7), pp. 965–979. doi: 10.1016/j.cell.2004.05.012.

- Harem, M., Kleiser, A. and Schuller, G. (1995) 'Responses of collicular neurons to acoustic motion in the horseshoe bat *Rhinolophus rouxi*', *Naturwissenschaften* 82, pp. 337–340.
- Harley, C. M., English, B. A. and Ritzmann, R. E. (2009) 'Characterization of obstacle negotiation behaviors in the cockroach, *Blaberus discoidalis*', *Journal of Experimental Biology*, 212(10), pp. 1463–1476. doi: 10.1242/jeb.028381.
- Harris, J. D. (1972) 'A florilegium of experiments on directional hearing.', *Acta oto-laryngologica. Supplementum*, 298, pp. 1–26.
- Harris, J. D. and Sergeant, R. L. (1971) 'Monaural/binaural minimum audible angles for a moving sound source', *Journal of Speech and Hearing Research*, 14(3).
- Hedwig, B. (2000) 'A highly sensitive opto-electronic system for the measurement of movements', *Journal of Neuroscience Methods*, 100(1–2), pp. 165–171. doi: 10.1016/S0165-0270(00)00255-7.
- Hedwig, B. (2000) 'Control of cricket stridulation by a command neuron: Efficacy depends on the behavioral state', *Journal of Neurophysiology*, 83(2), pp. 712–722. doi: 10.1152/jn.2000.83.2.712.
- Hedwig, B. (2014) 'Towards an Understanding of the Neural Basis of Acoustic Communication in Crickets', in Hedwig, B. (ed.) *Insect Hearing and Acoustic Communication*. New York: Springer, pp. 125–143.
- Hedwig, B. and Meyer, J. (1994) 'Auditory information processing in stridulating grasshoppers: Tympanic membrane vibrations and neurophysiology', *Journal of Comparative Physiology A*, 174(1), pp. 121–131. doi: 10.1007/BF00192013.
- Hedwig, B. and Poulet, J. F. A. (2004) 'Complex auditory behaviour emerges from simple reactive steering', *Nature*, 430, pp. 781–785. doi: 10.1038/nature02723.1.
- Hedwig, B. and Poulet, J. F. A. (2005) 'Mechanisms underlying phonotactic steering in the cricket *Gryllus bimaculatus* revealed with a fast trackball system.', *The Journal of experimental biology*, 208, pp. 915–27. doi: 10.1242/jeb.01452.
- Hedwig, B. and Stumpner, A. (2016) 'Central Neural Processing of Sound Signals in Insects', in *Insect Hearing*, pp. 177–214.

- Heffner, R. S. and Heffner, H. E. (1982) 'Hearing in the elephant (*Elephas maximus*): absolute sensitivity, frequency discrimination, and sound localization', *Journal of Comparative and Physiological Psychology*, 96(6), pp. 926–944.
- Heffner, R. S. and Heffner, H. E. (1992a) 'Hearing and sound localization in blind mole rats (*Spalax ehrenbergi*)', *Hearing Research*, 62(2), pp. 206–216. doi: 10.1016/0378-5955(92)90188-S.
- Heffner, R. S. and Heffner, H. E. (1992b) 'Hearing in large mammals: Sound-localization acuity in cattle (*Bos taurus*) and goats (*Capra hircus*).', *Journal of Comparative Psychology*, 106(2), pp. 107–113.
- Helversen, D. von and Rheinlaender, J. (1988) 'Interaural intensity and time discrimination in an unrestrained grasshopper: a tentative behavioural approach', 162, pp. 333–340.
- Hengstenberg, R. (1991) 'Gaze control in the blowfly *Calliphora*: a multisensory, two-stage integration process', *Seminars in Neuroscience*, 3(1), pp. 19–29. doi: 10.1016/1044-5765(91)90063-T.
- Hennig, R. M., Franz, A. and Stumpner, A. (2004) 'Processing of auditory information in insects', *Microscopy Research and Technique*, 63(6), pp. 351–374. doi: 10.1002/jemt.20052.
- Hirtenlehner, S., Römer, H. and Schmidt, A. K. D. (2014) 'Out of phase: Relevance of the medial septum for directional hearing and phonotaxis in the natural habitat of field crickets', *Journal of Comparative Physiology A*, 200(2), pp. 139–148. doi: 10.1007/s00359-013-0869-8.
- Honegger, H. -W *et al.* (1990) 'Morphology of antennal motoneurons in the brains of two crickets, *Gryllus bimaculatus* and *Gryllus campestris*', *Journal of Comparative Neurology*, 291(2), pp. 256–268. doi: 10.1002/cne.902910208.
- Honegger, H. W. (1981) 'A preliminary note on a new optomotor response in crickets: Antennal tracking of moving targets', *Journal of Comparative Physiology A*, 142(3), pp. 419–421. doi: 10.1007/BF00605454.
- Honegger, H. W. *et al.* (1984) 'A comparative study of neck muscle motor neurons in a cricket and a locust', *The Journal of Comparative Neurology*, 230(4), pp. 517–535. doi: 10.1002/cne.902300404.
- Horseman, B. G., Gebhardt, M. J. and Honegger, H. W. (1997) 'Involvement of the suboesophageal and thoracic ganglia in the control of antennal movements in crickets', *Journal of Comparative Physiology*

A, 181(3), pp. 195–204. doi: 10.1007/s003590050106.

Huber, F., Wohlers, D. W. and Moore, T. E. (1980) 'Auditory nerve and interneurone responses to natural sounds in several species of cicadas', *Physiological Entomology*, 5(1), pp. 25–45. doi: 10.1111/j.1365-3032.1980.tb00209.x.

Hughes, G. M. (1957) 'The co-ordination of insect movements: II. The effect of limb amputation and the cutting of commissures in the cockroach (*Blatta oiuentalis*)', *Journal of Experimental Biology*, 34(3), pp. 306–333. Available at: <http://jeb.biologists.org/content/34/3/306.short>.

Huston, S. J. and Jayaraman, V. (2011) 'Studying sensorimotor integration in insects', *Current Opinion in Neurobiology*, 21(4), pp. 527–534. doi: 10.1016/j.conb.2011.05.030.

Imaizumi, K. and Pollack, G. S. (1999) 'Neural coding of sound frequency by cricket auditory receptors', *Journal of Neuroscience*, 19(4), pp. 1508–1516. doi: 10.1523/jneurosci.19-04-01508.1999.

Imaizumi, K. and Pollack, G. S. (2001) 'Neural representation of sound amplitude by functionally different auditory receptors in crickets', *The Journal of the Acoustical Society of America*, 109(3), pp. 1247–1260. doi: 10.1121/1.1348004.

Imaizumi, K. and Pollack, G. S. (2005) 'Central projections of auditory receptor neurons of crickets', *Journal of Comparative Neurology*, 493(3), pp. 439–447. doi: 10.1002/cne.20756.

Jeram, S. *et al.* (1995) 'Structure of atympanate tibial organs in legs of the cave-living ensifera, *Troglophilus neglectus* (Gryllacridoidea, Raphidophoridae)', *Journal of Morphology*, 223(1), pp. 109–118. doi: 10.1002/jmor.1052230110.

Jia, F. Y. and Greenfield, M. D. (1997) 'When are good genes good? Variable outcomes of female choice in wax moths', *Proceedings of the Royal Society B: Biological Sciences*, 264(1384), pp. 1057–1063. doi: 10.1098/rspb.1997.0146.

Jonsson, T. *et al.* (2016) 'Auditory mechanics in a bush-cricket: Direct evidence of dual sound inputs in the pressure difference receiver', *Journal of the Royal Society Interface*, 13(122). doi: 10.1098/rsif.2016.0560.

Kamikouchi, A. *et al.* (2009) 'The neural basis of *Drosophila* gravity-sensing and hearing', *Nature*,

458(7235), pp. 165–171. doi: 10.1038/nature07810.

Keil, T. A. (1992) 'Fine structure of a developing insect olfactory organ: Morphogenesis of the silkworm antenna', *Microscopy Research and Technique*, 22(4), pp. 351–371. doi: 10.1002/jemt.1070220405.

Kevan, P. G. and Lane, M. A. (1985) 'Flower petal microtexture is a tactile cue for bees', *Proceedings of the National Academy of Sciences*, 82(14), pp. 4750–4752. doi: 10.1073/pnas.82.14.4750.

Klowden, M. J. (2013) *Physiological Systems in Insects*. 3rd edn. Amsterdam: Academic Press.

Knepper, M. and Hedwig, B. (1997) 'NEUROLAB, a PC-program for the processing of neurobiological data', *Computer Methods and Programs in Biomedicine*, 52(1), pp. 75–77. doi: 10.1016/S0169-2607(96)01781-6.

Knudsen, E. I., Blasdel, G. G. and Konishi, M. (1979) 'Sound localization by the barn owl (*Tyto alba*) measured with the search coil technique', *Journal of Comparative Physiology A*, 133(1), pp. 1–11. doi: 10.1007/BF00663105.

Kössl, M. and Boyan, G. S. (1998) 'Acoustic distortion products from the ear of a grasshopper', *The Journal of the Acoustical Society of America*, 104(1), p. 326. doi: 10.1121/1.423258.

Kostarakos, K. and Hedwig, B. (2012) 'Calling song recognition in female crickets: Temporal tuning of identified brain neurons matches behavior', 32(28), pp. 9601–9612. doi: 10.1523/JNEUROSCI.1170-12.2012.

Kostarakos, K. and Römer, H. (2010) 'Sound transmission and directional hearing in field crickets: Neurophysiological studies outdoors', *Journal of Comparative Physiology A*, 196(9), pp. 669–681. doi: 10.1007/s00359-010-0557-x.

Krause, A. F. and Dürr, V. (2012) 'Active tactile sampling by an insect in a step-climbing paradigm', *Frontiers in Behavioral Neuroscience*, 6(JUNE), pp. 1–17. doi: 10.3389/fnbeh.2012.00030.

Krause, A. F., Winkler, A. and Dürr, V. (2013) 'Central drive and proprioceptive control of antennal movements in the walking stick insect', *Journal of Physiology Paris*, 107(1–2), pp. 116–129. doi: 10.1016/j.jphysparis.2012.06.001.

Kriegbaum, H. (1989) 'Female choice in the Grasshopper *Chorthippus biguttulus*',

Naturwissenschaften, 76, pp. 81–82.

Kutsch, W. and Huber, F. (1990) 'Neural Basis of Song Production', in Huber, F., Moore, T. E., and Loher, W. (eds) *Cricket Behavior and Neurobiology*. New York: Cornell University Press, pp. 262–309.

Larsen, O. N., Kleindienst, H.-U. and Michelsen, A. (1989) 'Biophysical aspects of sound perception', in F. H., Moore, T. E., and Loher, W. (eds) *Cricket behavior and neurobiology*. Ithaca: Cornell University Press, pp. 364–390.

Larsen, O. N. and Michelsen, A. (1978) 'Biophysics of the ensiferan ear - III. The cricket ear as a four-input system', *Journal of Comparative Physiology A*, 123(3), pp. 217–227. doi: 10.1007/BF00656874.

Löhe, G. and Kleindienst, H.-U. (1994) 'The role of the medial septum in the acoustic trachea of the cricket *Gryllus bimaculatus* II . Influence on directionality of the auditory system', *Journal of Comparative Physiology A*, 174, pp. 601–606.

Loher, W. and Rence, B. (1978) 'The mating behavior of *Teleogryllus commodus* (Walker) and its central and peripheral control', *Zeitschrift für Tierpsychologie*, 46(3), pp. 225–259. doi: 10.1111/j.1439-0310.1978.tb01447.x.

Lv, M., Zhang, X. and Hedwig, B. (2020) 'Phonotactic steering and representation of directional information in the ascending auditory pathway of a cricket', *Journal of Neurophysiology*, 123(3), pp. 865–875. doi: 10.1152/jn.00737.2019.

Manley, G. A. (2012) 'Vertebrate hearing: Origin, evolution and functions', in Barth, F. G., Giampieri-Deutsch, P., and Klein, H.-D. (eds) *Sensory Perception*. Springer, pp. 23–40.

Marder, E. and Rehm, K. J. (2005) 'Development of central pattern generating circuits', *Current Opinion in Neurobiology*, 15(1), pp. 86–93. doi: 10.1016/j.conb.2005.01.011.

Mason, A. C., Oshinsky, M. L. and Hoy, R. R. (2001) 'Hyperacute directional hearing in a microscale auditory system', *Nature*, 410(6829), pp. 686–690. doi: 10.1038/35070564.

Matsumoto, Y. and Mizunami, M. (2000) 'Olfactory learning in the cricket *Gryllus bimaculatus*', *Journal of Experimental Biology*, 203(17), pp. 2581–2588. doi: 10.1242/jeb.203.17.2581.

May, M. L., Brodfuehrer, P. D. and Hoy, R. R. (1988) 'Kinematic and aerodynamic aspects of ultrasound-

induced negative phonotaxis in flying Australian field crickets (*Teleogryllus oceanicus*)', *Journal of Comparative Physiology A*, 164(2), pp. 243–249. doi: 10.1007/BF00603954.

Meier, T. and Reichert, H. (1990) 'Embryonic development and evolutionary origin of the orthopteran auditory organs', *Journal of Neurobiology*, 21(4), pp. 592–610. doi: 10.1002/neu.480210407.

Merlin, C., Gegear, R. J. and Reppert, S. M. (2009) 'Antennal circadian clocks coordinate sun compass orientation in migratory monarch butterflies', *Science*, 325(5948), pp. 100–1704. doi: 10.1126/science.1176221.Antennal.

Mhatre, N. and Robert, D. (2013) 'A tympanal insect ear exploits a critical oscillator for active amplification and tuning', *Current Biology*, 23(19), pp. 1952–1957. doi: 10.1016/j.cub.2013.08.028.

Michelsen, A. (1994) 'Directional hearing in crickets and other small animals', *Fortschr. Zoologie*, 39, pp. 195–207.

Michelsen, A. (1998) 'The tuned cricket', *News Physiol. Sci.*, 13, pp. 32–38.

Michelsen, A. and Löhe, G. (1995) 'Tuned directionality in cricket ears', *Nature*, p. 639. doi: 10.1038/375639a0.

Michelsen, A., Popov, A. V. and Lewis, B. (1994) 'Physics of directional hearing in the cricket *Gryllus bimaculatus*', *Journal of Comparative Physiology A*, 175(2), pp. 153–164. doi: 10.1007/BF00215111.

Miller, L. A. (1970) 'Structure of the green lacewing tympanal organ (*Chrysopa carnea*, Neuroptera)', *Journal of Morphology*, 131, pp. 359–382.

Miller, P. L. (1972) 'Swimming in mantids', *Journal of Entomology Series A, General Entomology*, 46(2), pp. 91–97. doi: 10.1111/j.1365-3032.1972.tb00113.x.

Mills, A. W. (1958) 'On the minimum audible angle', *Journal of the Acoustical Society of America*, 30(4), pp. 237–246. doi: 10.1121/1.1909553.

Mizunami, M. and Matsumoto, Y. (2017) 'Learning and memory', in Horch, H. W. et al. (eds) *The Cricket as a Model Organism*. Tokyo: Springer Japan, pp. 129–140.

Moiseff, A. and Hoy, R. (1983) 'Sensitivity to ultrasound in an identified auditory interneuron in the cricket: a possible neural link to phonotactic behavior', *Journal of Comparative Physiology A*, 152(2),

pp. 155–167. doi: 10.1007/BF00611181.

Moiseff, A., Pollack, G. S. and Hoy, R. R. (1978) 'Steering responses of flying crickets to sound and ultrasound: Mate attraction and predator avoidance', *Proceedings of the National Academy of Sciences*, 75(8), pp. 4052--4056. doi: 10.1073/pnas.75.8.4052.

Molina, J. and Stumpner, A. (2005) 'Effects of pharmacological treatment and photoinactivation on the directional responses of an insect neuron', *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, 303(12), pp. 1085–1103. doi: 10.1002/jez.a.228.

Mongeau, J. M. *et al.* (2013) 'Locomotion-and mechanics-mediated tactile sensing: Antenna reconfiguration simplifies control during high-speed navigation in cockroaches', *Journal of Experimental Biology*, 216(24), pp. 4530–4541. doi: 10.1242/jeb.083477.

Mora, E. C. *et al.* (2015) 'Unexpected dynamic up-tuning of auditory organs in day-flying moths', *Journal of Comparative Physiology A*, pp. 657–666. doi: 10.1007/s00359-015-1009-4.

Morse, P. M. and Ingard, K. U. (1968) *Theoretical Acoustics*. New York: McGraw-Hill.

Murphey, R. K. and Zaretsky, M. D. (1972) 'Orientation to calling song by female crickets, *Scapsipedus marginatus* (Gryllidae).', *The Journal of experimental biology*, 56(2), pp. 335–352.

Nabatiyan, A. *et al.* (2003) 'Temporal pattern recognition based on instantaneous spike rate coding in a simple auditory system', *Journal of Neurophysiology*, 90(4), pp. 2484–2493. doi: 10.1152/jn.00259.2003.

Nadrowski, B. *et al.* (2011) 'Antennal hearing in insects - New findings, new questions', *Hearing Research*, 273(1–2), pp. 7–13. doi: 10.1016/j.heares.2010.03.092.

Nakagawa, H. and Hongjian, K. (2010) 'Collision-sensitive neurons in the optic tectum of the bullfrog, *Rana catesbeiana*', *Journal of Neurophysiology*, 104(5), pp. 2487–2499. doi: 10.1152/jn.01055.2009.

Nakano, R. *et al.* (2009) 'Private ultrasonic whispering in moths', *Communicative and Integrative Biology*, 2(2), pp. 123–126. doi: 10.1073/pnas.0804056105.123.

Nocke, H. (1972) 'Physiological aspects of sound communication in crickets (*Gryllus campestris* L.)', *Journal of Comparative Physiology A*, 80(2), pp. 141–162. doi: 10.1007/BF00696487.

Nolen, T. G. and Hoy, R. R. (1984) 'Initiation of behavior by single neurons: the role of behavioral context', *Science*, 226(4677), pp. 992–994.

Okada, J. and Toh, Y. (2004) 'Spatio-temporal patterns of antennal movements in the searching cockroach', *Journal of Experimental Biology*, 207(21), pp. 3693–3706. doi: 10.1242/jeb.01201.

Okada, J. and Toh, Y. (2006) 'Active tactile sensing for localization of objects by the cockroach antenna', *Journal of Comparative Physiology A*, 192(7), pp. 715–726. doi: 10.1007/s00359-006-0106-9.

Olberg, R. M. *et al.* (2007) 'Eye movements and target fixation during dragonfly prey-interception flights', *Journal of Comparative Physiology A*, 193(7), pp. 685–693. doi: 10.1007/s00359-007-0223-0.

Oldfield, B. P. (1980) 'Accuracy of orientation in female crickets, *Teleogryllus oceanicus* (Gryllidae): Dependence on song spectrum', *Journal of Comparative Physiology A*, 141, pp. 93–99.

Oldfield, B. P. (1982) 'Tonotopic organisation of auditory receptors in Tettigoniidae (Orthoptera: Ensifera)', *Journal of Comparative Physiology A*, 147(4), pp. 461–469. doi: 10.1007/BF00612011.

Oldfield, B. P. (1984) 'Physiology of auditory receptors in two species of Tettigoniidae (Orthoptera: Ensifera) - Alternative tonotopic organisations of the auditory organ', *Journal of Comparative Physiology A*, 155(5), pp. 689–696. doi: 10.1007/BF00610855.

Oldfield, B. P., Kleindienst, H. U. and Huber, F. (1986) 'Physiology and tonotopic organization of auditory receptors in the cricket *Gryllus bimaculatus* DeGeer', *Journal of Comparative Physiology A*, 159(4), pp. 457–464. doi: 10.1007/BF00604165.

Owaki, D. *et al.* (2021) 'Leg amputation modifies coordinated activation of the middle leg muscles in the cricket *Gryllus bimaculatus*', *Scientific Reports*, 11(1), pp. 1–12. doi: 10.1038/s41598-020-79319-6.

Payne, R. S., Roeder, K. D. and Wallman, J. (1966) 'Directional sensitivity of the ears of noctuid moths.', *The Journal of experimental biology*, 44, pp. 17–31.

Pelletier, Y. and McLeod, C. D. (1994) 'Obstacle perception by insect antennae during terrestrial locomotion', *Physiological Entomology*, 19(4), pp. 360–362. doi: 10.1111/j.1365-

3032.1994.tb01063.x.

Perrott, D. R. and Musicant, A. D. (1977) 'Minimum auditory movement angle: Binaural localization of moving sound sources', *Journal of the Acoustical Society of America*, 62(6), pp. 1463–1466. doi: 10.1121/1.381675.

Petrou, G. and Webb, B. (2012) 'Detailed tracking of body and leg movements of a freely walking female cricket during phonotaxis', *Journal of Neuroscience Methods*, 203, pp. 56–68. doi: 10.1016/j.jneumeth.2011.09.011.

Plotnick, R. E. and Smith, D. M. (2012) 'Exceptionally preserved fossil insect ears from the Eocene green river formation of colorado', *Journal of Paleontology*, 86(1), pp. 19–24.

Pollack, G. S. and Faulkes, Z. (1998) 'Representation of behaviorally relevant sound frequencies by auditory receptors in the cricket *Teleogryllus oceanicus*', *Journal of Experimental Biology*, 201(1), pp. 155–163. doi: 10.1242/jeb.201.1.155.

Pollack, G. S. and Hoy, R. (1979) 'Temporal pattern as a cue for species-specific calling recognition in crickets', *Science*, 204(4391), pp. 429–432.

Pollack, G. S. and Martins, R. (2007) 'Flight and hearing: ultrasound sensitivity differs between flight-capable and flight-incapable morphs of a wing-dimorphic cricket species', *The Journal of experimental biology*, 210(18), pp. 3160–4. doi: 10.1242/jeb.008136.

Poulet, J. F. A. and Hedwig, B. (2005) 'Auditory orientation in crickets: Pattern recognition controls reactive steering', *PNAS*, 102(43), pp. 15665–15669.

Poulet, J. F. A. and Hedwig, B. (2006) 'The cellular basis of a corollary discharge', *Science*, 311(5760), pp. 518–522.

Prete, F. R. *et al.* (2012) 'Visual stimuli that elicit visual tracking, approaching and striking behavior from an unusual praying mantis, *Euchomenella macrops* (Insecta: Mantodea)', *Journal of Insect Physiology*, 58(5), pp. 648–659. doi: 10.1016/j.jinsphys.2012.01.018.

Preuss, T. *et al.* (2006) 'Neural representation of object approach in a decision-making motor circuit', *Journal of Neuroscience*, 26(13), pp. 3454–3464. doi: 10.1523/JNEUROSCI.5259-05.2006.

- Quinlan, M. C. and Gibbs, A. G. (2006) 'Discontinuous gas exchange in insects', *Respiratory Physiology and Neurobiology*, 154(1–2), pp. 18–29. doi: 10.1016/j.resp.2006.04.004.
- Rauschecker, J. P. and Harris, L. R. (1989) 'Auditory and visual neurons in the cat's superior colliculus selective for the direction of apparent motion stimuli', *Brain Research*, 490(1), pp. 56–63. doi: 10.1016/0006-8993(89)90430-7.
- Renaud, D. L. and Popper, A. N. (1975) 'Sound localization by the bottlenose porpoise *Tursiops truncatus*', *Journal of Experimental Biology*, 63(3), pp. 569–585.
- Rheinlaender, J. and Blätgen, G. (1982) 'The precision of auditory lateralization in the cricket, *Gryllus bimaculatus*', *Physiological Entomology*, 7, pp. 209–218.
- Rheinlaender, J. and Römer, H. (1990) 'Acoustic cues for sound localization and spacing in Orthopteran insect', in Bailey, W. J. and Rentz, D. C. F. (eds) *The Tettigoniidae, biology, systematics and evolution*. Bathurst: Crawford House Press.
- Rheinlaender, J., Shen, J. X. and Römer, H. (2006) 'Auditory lateralization in bushcrickets: A new dichotic paradigm', *Journal of Comparative Physiology A*, 192(4), pp. 389–397. doi: 10.1007/s00359-005-0078-1.
- Ritchie, M. G., Couzin, I. D. and Snedden, W. A. (1995) 'What's in a song? Female bushcrickets discriminate against the song of older males', *Proceedings of the Royal Society of London B*, 262, pp. 21–17.
- Ritzmann, R. E. *et al.* (2012) 'Deciding which way to go: How do insects alter movements to negotiate barriers?', *Frontiers in Neuroscience*, 6(JULY), pp. 1–10. doi: 10.3389/fnins.2012.00097.
- Robert, D. (2005) 'Directional Hearing in Insects', in Arthur N, P. and Fay, R. R. (eds) *Sound Source Localization*. New York: Springer, pp. 6–35.
- Robert, D. (2009) 'Insect bioacoustics: Mosquitoes make an effort to listen to each other', *Current Biology*, 19(11), pp. 446–449. doi: 10.1016/j.cub.2009.04.021.
- Robert, D., Miles, R. N. and Hoy, R. R. (1996) 'Directional hearing by mechanical coupling in the parasitoid fly *Ormia ochracea*', *Journal of comparative physiology A*, 179, pp. 29–44. doi:

10.1007/BF00193432.

Robinson, D., Rheinlaender, J. and Hartley, J. C. (1986) 'Temporal parameters of male-female sound communication in *Leptophyes punctatissima*', *Physiological Entomology*, 11(3), pp. 317–323. doi: 10.1111/j.1365-3032.1986.tb00419.x.

Roeder, K. D. (1998) *Nerve Cells and Insect Behavior*. Revised. Cambridge, MA: Harvard University Press.

Römer, H., Hedwig, B. and Ott, S. R. (2002) 'Contralateral inhibition as a sensory bias: The neural basis for a female preference in a synchronously calling bushcricket, *Mecopoda elongata*', *European Journal of Neuroscience*, 15(10), pp. 1655–1662. doi: 10.1046/j.1460-9568.2002.02003.x.

Römer, H. and Krusch, M. (2000) 'A gain-control mechanism for processing of chorus sounds in the afferent auditory pathway of the bushcricket *Tettigonia viridissima* (Orthoptera; Tettigoniidae)', *Journal of Comparative Physiology A*, 186, pp. 181–91. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10707316>.

Römer, H., Rheinlaender, J. and Dronse, R. (1981) 'Intracellular studies on auditory processing in the metathoracic ganglion of the locust', *Journal of Comparative Physiology A*, 144, pp. 305–312.

Ronacher, B. and Hennig, R. M. (2004) 'Neuronal adaptation improves the recognition of temporal patterns in a grasshopper', *Journal of Comparative Physiology A*, 190(4), pp. 311–319. doi: 10.1007/s00359-004-0498-3.

Roy Khurana, T. and Sane, S. P. (2016) 'Airflow and optic flow mediate antennal positioning in flying honeybees', *eLife*, 5(APRIL2016), pp. 1–20. doi: 10.7554/eLife.14449.

Rust, J., Stumpner, A. and Gottwald, J. (1999) 'Singing and hearing in a Tertiary bushcricket', *Nature*, 399(6737), p. 650. doi: 10.1038/21356.

Ryan, M. J. (1988) 'Constraints and patterns in the evolution of anuran acoustic communication', in Fritzsche, B. (ed.) *The Evolution of The Amphibian Auditory System*. New York: Wiley, pp. 637–677.

Ryan, M. J. and Keddy-Hector, A. (1992) 'Directional patterns of female mate choice and the role of sensory biases', *The American Naturalist*, 139, pp. 4–35.

- Sabourin, P., Gottlieb, H. and Pollack, G. S. (2008) 'Carrier-dependent temporal processing in an auditory interneuron', *The Journal of the Acoustical Society of America*, 123(5), pp. 2910–2917. doi: 10.1121/1.2897025.
- Sakura, M. and Aonuma, H. (2013) 'Aggressive behavior in the antennectomized male cricket *Gryllus bimaculatus*', *Journal of Experimental Biology*, 216(12), pp. 2221–2228. doi: 10.1242/jeb.079400.
- Sane, S. P. *et al.* (2007) 'Antennal mechanosensors mediate flight control in moths', *Science*, 315(5813), pp. 863–866. doi: 10.1126/science.1133598.
- Sarmiento-Ponce, E. J., Rogers, S. and Hedwig, B. (2021) 'Does the choosiness of female crickets change as they age?', *Journal of Experimental Biology*, 224(11). doi: 10.1242/JEB.241802.
- Sarria-S, F. A. *et al.* (2017) 'Non-invasive biophysical measurement of travelling waves in the insect inner ear', *Royal Society Open Science*, 4(5), pp. 1–11. doi: 10.1098/rsos.170171.
- Schildberger, K. (1984) 'Temporal selectivity of identified auditory neurons in the cricket brain', *Journal of Comparative Physiology A*, 155(2), pp. 171–185. doi: 10.1007/BF00612635.
- Schildberger, K. and Hörner, M. (1988) 'The function of auditory neurons in cricket phonotaxis I. Influence of hyperpolarization of identified neurons on sound localization', *Journal of Comparative Physiology A*, 163, pp. 621–631. doi: 10.1007/BF00603847.
- Schmidt, A. K. D. and Römer, H. (2013) 'Diversity of acoustic tracheal system and its role for directional hearing in crickets', *Frontiers in Zoology*, 10(1), pp. 1–9. doi: 10.1186/1742-9994-10-61.
- Schmidt, A. K. D. and Römer, H. (2016) 'Functional relevance of acoustic tracheal design in directional hearing in crickets', *Journal of Experimental Biology*, 219(20), pp. 3294–3300. doi: 10.1242/jeb.145524.
- Schmitz, B., Scharstein, H. and Wendler, G. (1982) 'Phonotaxis in *Gryllus campestris* L. (Orthoptera, Gryllidae) I. Mechanism of acoustic orientation in intact female crickets', *Journal of Comparative Physiology A*, 148, pp. 431–444.
- Schöneich, S. and Hedwig, B. (2010) 'Hyperacute directional hearing and phonotactic steering in the cricket (*Gryllus bimaculatus* deGeer)', *PLOS ONE*, 5(12). doi: 10.1371/journal.pone.0015141.

- Schöneich, S. and Hedwig, B. (2011) 'Neural basis of singing in crickets: Central pattern generation in abdominal ganglia', *Naturwissenschaften*, 98(12), pp. 1069–1073. doi: 10.1007/s00114-011-0857-1.
- Schöneich, S. and Hedwig, B. (2012) 'Cellular basis for singing motor pattern generation in the field cricket (*Gryllus bimaculatus* DeGeer)', *Brain and Behavior*, 2(6), pp. 707–725. doi: 10.1002/brb3.89.
- Schöneich, S., Kostarakos, K. and Hedwig, B. (2015) 'An auditory feature detection circuit for sound pattern recognition', *Science Advances*, 1(8), pp. 1–8. doi: 10.1126/sciadv.1500325.
- Schul, J. (1997) 'Neuronal basis of phonotactic behaviour in *Tettigonia viridissima*: processing of behaviourally relevant signals by auditory afferents and thoracic interneurons', *Journal of Comparative Physiology A*, 180, pp. 573–583.
- Schütz, C. and Dürr, V. (2011) 'Active tactile exploration for adaptive locomotion in the stick insect', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366(1581), pp. 2996–3005. doi: 10.1098/rstb.2011.0126.
- Selverston, A. I., Kleindienst, H.-U. and Huber, F. (1985) 'Synaptic connectivity between cricket auditory interneurons studied by selective photoactivation', *The Journal of Neuroscience*, 5(5), pp. 1283–1292.
- Shen, J. X. *et al.* (2008) 'Ultrasonic frogs show hyperacute phonotaxis to female courtship calls', *Nature*, 453(7197), pp. 914–916. doi: 10.1038/nature06719.
- Simmons, N. B. *et al.* (2008) 'Primitive Early Eocene bat from Wyoming and the evolution of flight and echolocation', *Nature*, 451(7180), pp. 818–821. doi: 10.1038/nature06549.
- Sláma, K. (1988) 'A new look at insect respiration', *The Biological Bulletin*, 175(2), pp. 289–300. doi: 10.2307/1541570.
- Spitzer, M. W. and Semple, M. N. (2000) 'Interaural phase coding in auditory midbrain: Influence of dynamic stimulus features', *Science*, 165(1979), pp. 721–724.
- Staudacher, E. (1998) 'Distribution and morphology of descending brain neurons in the cricket *Gryllus bimaculatus*', *Cell and Tissue Research*, 294(1), pp. 187–202. doi: 10.1007/s004410051169.
- Staudacher, E. M. (2001) 'Sensory responses of descending brain neurons in the walking cricket,

Gryllus bimaculatus', *Journal of Comparative Physiology A*, 187(1), pp. 1–17. doi: 10.1007/s003590000171.

Staudacher, E. M., Gebhardt, M. and Dürr, V. (2005) 'Antennal movements and mechanoreception: Neurobiology of active tactile sensors', *Advances in Insect Physiology*. doi: 10.1016/S0065-2806(05)32002-9.

Staudacher, E. and Schildberger, K. (1998) 'Gating of sensory responses of descending brain neurones during walking in crickets', *Journal of Experimental Biology*, 201(4), pp. 559–572. doi: 10.1242/jeb.201.4.559.

Stout, J. F., DeHaan, C. H. and McGhee, R. W. (1983) 'Attractiveness of the male *Acheta domestica* calling song to females', *Journal of Comparative Physiology A*, 153(4), pp. 509–521. doi: 10.1007/bf00612605.

Strauß, J. and Lakes-Harlan, R. (2014) 'Evolutionary and Phylogenetic Origins of Tympanal Hearing Organs in Insects', in Hedwig, B. (ed.) *Insect Hearing and Acoustic Communication*. New York: Springer, pp. 11–26.

Stumpner, A. (1996) 'Complete morphology of sound-activated plurisegmental interneurons in a bushcricket', *Göttingen Neurobiology Report 1996*, p. A167.

Stumpner, A. (1997) 'An auditory interneurone tuned to the male song frequency in the duetting bushcricket *Ancistrura nigrovittata* (Orthoptera, Phaneropteridae)', *The Journal of Experimental Biology*, 200, pp. 1089–1011.

Stumpner, A. (1999) 'An interneurone of unusual morphology is tuned to the female song frequency in the bushcricket *Ancistrura nigrovittata* (Orthoptera, Phaneropteridae)', *Journal of Experimental Biology*, 202(15), pp. 2071–2081. doi: 10.1242/jeb.202.15.2071.

Stumpner, A. and Meyer, S. (2001) 'Songs and the function of song elements in four duetting bushcricket species (Ensifera, Phaneropteridae, Barbitistes)', *Journal of Insect Behavior*, 14(4), pp. 511–534. doi: 10.1023/A:1011176106943.

Sun, H. and Frost, B. J. (1998) 'Computation of different optical variables of looming objects in pigeon

nucleus rotundus neurons', *Nature Neuroscience*, 1(4), pp. 296–303. doi: 10.1038/1110.

Tougaard, J. (1998) 'Detection of short pure-tone stimuli in the noctuid ear: What are temporal integration and integration time all about?', *Journal of Comparative Physiology A*, 183(5), pp. 563–572. doi: 10.1007/s003590050282.

Tully, T. and Quinn, W. G. (1985) 'Classical conditioning and retention in normal and mutant *Drosophila melanogaster*', *Journal of Comparative Physiology A*, 157(2), pp. 263–277. doi: 10.1007/BF01350033.

Ulagaraj, S. M. and Walker, T. J. (1973) 'Phonotaxis of crickets in flight: Attraction of male and female crickets to male calling songs', *Science*, 182(4118), pp. 1278–1279. doi: 10.1126/science.182.4118.1278.

Veitch, D. *et al.* (2021) 'A narrow ear canal reduces sound velocity to create additional acoustic inputs in a microscale insect ear', *Proceedings of the National Academy of Sciences of the United States of America*, 118(10). doi: 10.1073/pnas.2017281118.

Wagner, H. and Takahashi, T. (1990) 'Neurons in the midbrain of the barn owl are sensitive to the direction of apparent acoustic motion', *Naturwissenschaften*, 77(9), pp. 439–442. doi: 10.1007/BF01135947.

Wagner, H. and Takahashi, T. (1992) 'Influence of temporal cues on acoustic motion-direction sensitivity of auditory neurons in the owl', *Journal of Neurophysiology*, 68(6), pp. 2063–2076. doi: 10.1152/jn.1992.68.6.2063.

Wagner, W. E. (1998) 'Measuring female mating preferences', *Animal Behaviour*, 55(4), pp. 1029–1042. doi: 10.1006/anbe.1997.0635.

Wagner, W. E. (2011) 'Direct benefits and the evolution of female mating preferences: Conceptual Problems, Potential Solutions, and a Field Cricket', in *Advances in the study of Behavior Vol 43*. Amsterdam: Elsevier, pp. 273–319.

Wasserthal, L. T. (1996) 'Interaction of circulation and tracheal ventilation in holometabolous insects', *Advances in Insect Physiology*, 26, pp. 297–351, doi: 10.1016/S0065-2806(08)60033-8.

Weber, T., Thorson, J. and Huber, F. (1981) 'Auditory behavior of the cricket - I. Dynamics of

compensated walking and discrimination paradigms on the Kramer treadmill', *Journal of Comparative Physiology A*, 141(2), pp. 215–232. doi: 10.1007/BF01342668.

Weis-Fogh, T. (1964) 'Functional Design of the Tracheal System of Flying Insects as Compared with the Avian Lung', *Journal of Experimental Biology*, 41(2), pp. 207–227. doi: 10.1242/jeb.41.2.207.

Wendler, G. *et al.* (1980) 'Analysis of the acoustic orientation behavior in crickets (*Gryllus campestris* L.)', *Naturwissenschaften*, 67(2), pp. 99–101. doi: 10.1007/BF01054701.

Wendler, G. and Löhe, G. (1993) 'The role of the medial septum in the acoustic trachea of the cricket *Gryllus bimaculatus* - I. Importance for efficient phonotaxis', *Journal of Comparative Physiology A*, 173(5), pp. 557–564. doi: 10.1007/BF00197764.

Westneat, M. W. *et al.* (2003) 'Tracheal respiration in insects visualized with synchrotron x-ray imaging', *Science*, 299(5606), pp. 558–560. doi: 10.1126/science.1078008.

Wiese, K. and Eilts-Grimm, K. (1985) 'Functional potential of recurrent lateral inhibition in cricket audition', *Acoustic and vibrational communication in insects: Proceedings from the XVII International Congress of Entomology held at the University of Hamburg, August 1984 / Klaus Kalmring and Norbert Elsner (eds.)*. Berlin [W. Ger.]: P. Parey, 1985.

Wigglesworth, V. B. (1959) 'The Histology of the Nervous System of an Insect, *Rhodnius prolixus* (Hemiptera) II. The central ganglia', *Journal of Cell Science*, 3(50), pp. 299–313. doi: 10.1242/jcs.s3-100.50.299.

Wilson, W. W. and O'Neill, W. E. (1998) 'Auditory motion induces directionally dependent receptive field shifts in inferior colliculus neurons', *Journal of Neurophysiology*, 79(4), pp. 2040–2062. doi: 10.1152/jn.1998.79.4.2040.

Windmill, J. F. C. *et al.* (2006) 'Keeping up with bats: Dynamic auditory tuning in a moth', *Current Biology*, 16(24), pp. 2418–2423. doi: 10.1016/j.cub.2006.09.066.

Windmill, J. F. C. and Jackson, J. C. (2016) 'Mechanical Specializations of Insect Ears', in Pollack, G. S. *et al.* (eds) *Insect Hearing*. Switzerland: Springer International Publishing, pp. 156–176.

Witney, A. G. and Hedwig, B. (2011) 'Kinematics of phonotactic steering in the walking cricket *Gryllus*

bimaculatus (de Geer)', *The Journal of experimental Biology*, 214, pp. 69–79. doi: 10.1242/jeb.044800.

Wohlers, D. W. and Huber, F. (1982) 'Processing of sound signals by six types of neurons in the prothoracic ganglion of the cricket, *Gryllus campestris* L.', *Journal of Comparative Physiology A*, 146(2), pp. 161–173. doi: 10.1007/BF00610234.

Wohlers, D. W. and Huber, F. (1985) 'Topographical organization of the auditory pathway within the prothoracic ganglion of the cricket *Gryllus campestris* L.', *Cell and Tissue Research*, 239(3), pp. 555–565. doi: 10.1007/BF00219234.

Wytenbach, R. A. and Hoy, R. R. (1997) 'Spatial acuity of ultrasound hearing in flying crickets', *The Journal of Experimental Biology*, 200, pp. 1999–2006.

Yack, J. E. (2004) 'The structure and function of auditory chordotonal organs in insects', *Microscopy Research and Technique*, 63(6), pp. 315–337. doi: 10.1002/jemt.20051.

Yack, J. E. and Fullard, J. H. (1993) 'What Is An Insect Ear', *Annals of the Entomological Society of America*, pp. 677–682.

Yager, D. D. (1999) 'Structure, development, and evolution of insect auditory systems', *Microscopy Research and Technique*, 47(6), pp. 380–400. doi: 10.1002/(SICI)1097-0029(19991215)47:6<380::AID-JEMT3>3.0.CO;2-P.

Yager, D. D. and Hoy, R. R. (1986) 'The cyclopean ear: A new sense for the praying mantis', *Science*, 231(4739), pp. 727–729. doi: 10.1126/science.3945806.

Yager, D. D. and Hoy, R. R. (1987) 'The midline metathoracic ear of the praying mantis, *Mantis religiosa*', *Cell Tissue Research*, 250, pp. 531–541.

Yager, D. D. and Hoy, R. R. (1989) 'Audition in the praying mantis, *Mantis religiosa* L.: Identification of an interneuron mediating ultrasonic hearing', *Journal of Comparative Physiology A*, 165(4), pp. 471–493. doi: 10.1007/BF00611236.

Yager, D. D., May, M. L. and Fenton, M. B. (1990) 'Ultrasound-triggered, flight-gated evasive maneuvers in the praying mantis *Parasphendale agrionina*. I. Free flight.', *The Journal of experimental biology*, 152(1), pp. 17–39. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/2230635>.

- Yamawaki, Y. *et al.* (2011) 'Coordinated movements of the head and body during orienting behaviour in the praying mantis *Tenodera aridifolia*', *Journal of Insect Physiology*, 57(7), pp. 1010–1016. doi: 10.1016/j.jinsphys.2011.04.018.
- Yin, T. C. T. and Kuwada, S. (1983) 'Binaural interaction in low-frequency neurons in inferior colliculus of the cat. III. Effects of changing frequency', *Journal of Neurophysiology*, 50(4), pp. 1020–1042. doi: 10.1152/jn.1983.50.4.1020.
- Young, D. and Ball, E. (1974) 'Structure and development of the auditory system in the prothoracic leg of the cricket *Teleogryllus commodus* (Walker) - I. Adult structure', *Zeitschrift für Zellforschung und mikroskopische Anatomie*, 147(3), pp. 293–312. doi: 10.1007/BF00307466.
- Zimmermann, U., Rheinlaender, J. and Robinson, D. (1989) 'Cues for male phonotaxis in the duetting bushcricket *Leptophyes punctatissima*', *Journal of Comparative Physiology A*, 164(5), pp. 621–628. doi: 10.1007/BF00614504.
- Zorović, M. and Hedwig, B. (2011) 'Processing of species-specific auditory patterns in the cricket brain by ascending, local, and descending neurons during standing and walking', *Journal of Neurophysiology*, 105(5), pp. 2181–2194. doi: 10.1152/jn.00416.2010.
- Zufall, F., Schmitt, M. and Menzel, R. (1989) 'Spectral and polarized light sensitivity of photoreceptors in the compound eye of the cricket (*Gryllus bimaculatus*)', *Journal of Comparative Physiology A*, 164(5), pp. 597–608. doi: 10.1007/BF00614502.
- Zurek, D. B. and Gilbert, C. (2014) 'Static antennae act as locomotory guides that compensate for visual motion blur in a diurnal, keen-eyed predator', *Proceedings of the Royal Society B: Biological Sciences*, 281(1779). doi: 10.1098/rspb.2013.3072.