ORIGINAL PAPER

Spatial orientation in the bushcricket *Leptophyes punctatissima* (Phaneropterinae; Orthoptera): III. Peripheral directionality and central nervous processing of spatial cues

Konstantinos Kostarakos · Jürgen Rheinlaender · Heiner Römer

Abstract We examined peripheral and central nervous cues underlying the ability of the bushcricket *Leptophyes punctatissima* to orient to elevated and depressed sound sources broadcasting the female acoustic reply. The peripheral spatial directionality of the ear was measured physiologically using monaural preparations of an auditory interneuron (T-fibre). In the azimuth, maximal interaural intensity differences of 18 dB occur between ipsi- and contralateral stimulation. With increasing elevation or depression of the sound sources, IIDs decrease systematically and reach zero with the source exactly above or below the preparation. Bilateral, simultaneous recordings of the activity of the pair of interneurons allowed determining the binaural discharge differences which occur in response to the extremely short (1 ms) female reply. These discharge differences are large (four action potentials/stimulus) and reliable in the azimuth with lateral stimulation, and decrease gradually with more frontal stimulation. With elevation and depression of sound sources these differences again decrease to one action potential/stimulus at 60° or 75° elevation, and lateral stimulus angles of about 60°. We also calculated the reliability with which a receiver could correctly determine the location of the sound source. We discuss these quantitative measures in relation to the spatial phonotactic behaviour of male *L. punctatissima*.

K. Kostarakos · J. Rheinlaender · H. Römer (✉)
Department of Zoology, Karl-Franzens-University,
8010 Graz, Austria
e-mail: heinrich.roemer@uni-graz.at

Abbreviations
IID   Interaural intensity difference
SPL   Sound pressure level

Introduction

Sound localization in the azimuth has been well studied in both vertebrates and invertebrates in terms of behaviour, the biophysics of the ears, and peripheral and central processing (review Pollack 2000; Gerhardt and Huber 2002). Spatial hearing, however, i.e. the localization of a sound source in the horizontal and vertical planes, has been restricted with only few exceptions to some selected vertebrates and owls. This is somewhat surprising given the fact that most hearing animals live in a complex, three-dimensional habitat where spatial hearing should be of great importance.

In humans and mammals, the particular shape of the external pinnae provides important information for the vertical orientation of a sound source because they act as spatially dependent filters for sound (Butler and Belendiuk 1977; Blauert 1983; Fuzessery 1996; Wotton and Simmons 2000; Popper and Fay 2005). As a result of this filtering, spectral cues are created that humans and other mammals appear to use in localizing the elevation or depression of a source. In owls, ear asymmetries provide the basis for their remarkable spatial acuity (Knudsen and Konishi 1979; Konishi 2003). The assumption, however, that these external ear structures are essential for spatial hearing has been disproved by Gerhardt and Rheinlaender (1982) when demonstrating that green tree frogs *Hyla cinerea* readily localize an elevated sound source without pinnae or similar anatomical structures.

The other large taxon where hearing has evolved many times independently are insects (Hoy and Robert 1996), which are also confronted with the problem that a sound source has to be localized in both azimuth and elevation. Until recently, however, there were only three studies addressing spatial hearing in insects. Payne et al. (1966) and Wyttenbach and Hoy (1997) investigated the spatial acuity of ultrasound hearing in moths and crickets in the context of bat avoidance during flight, and in a behavioural study Müller and Robert (2001) demonstrated the remarkable phonotactic accuracy of the parasitoid fly *Ormia ochracea.* These studies are concerned either with the ability of prey to localize a predator, or a parasitoid to locate its host.

In the context of intraspecific communication, we studied the ability of male bushcricket *Leptophyes punctatissima* to orient to elevated and depressed sound sources broadcasting simulated female responses (Rheinlaender et al. 2007). Males orientated in an artificial grid system, and all males tested reached the three speaker positions (one in the horizontal plane, one elevated by 45°, one depressed by 45° relative to the starting position) with only little deviation from the shortest possible path. This remarkable spatial orientation was achieved although the insects have no specialized external ear structures such as pinnae in mammals.

We further quantified the behavioural performance of males on a walking belt, by broadcasting the acoustic female responses from one of five speakers, elevated by 0°, 30°, 60°, 75°, and 90° above the male (Ofner et al. 2007). We hypothesized that with increasing elevation of the sound source the available directional cues would decrease, which should be detectable in quantitative measures of the phonotactic performance. This was indeed the case: with increasing speaker elevation, the meandering of males increased, and the ratio of the ideal path length to the actual path length decreased. The same was true for the correlation between stimulus angle and turn angle, and there were more turns to the wrong side with increasing speaker elevation. Most males performed phonotaxis with a high acuity up to an elevation of 60°. Remarkably, individuals varied strongly in their performance especially at a source elevation of 75°, where some were still very accurate in their approach whereas the acuity of others decreased rapidly.

The present paper is the last one in this series on spatial localization of the bushcricket *L. punctatissima.* We present for the first time data both on the directionality of the ear and on the central nervous processing of spatial information. Since the behavioural experiments on the walking compensator and the neurophysiology of central nervous processing have been performed under identical conditions, we will be able to directly correlate the degree of behavioural performance with the amount of spatial information available for the orienting male.

Materials and methods

Animals

A total of 25 male *L. punctatissima* (Tettigoniidae; Phaneropterinae) were used for neurophysiological experiments. They were captured as nymphs from wild stock locations in Nordkirchen (Nordrhein-Westfalen, Germany), and were reared to adulthood at the Institute of Zoology in Graz (Austria), where their favoured plant food (*Urtica dioica, Heracleum spondylium*, *Cirsium vulgare*) and water were supplied ad libitum. For experiments individuals aged between 1 and 6 weeks after the final moult were used.

Experimental set-up

The experimental set-up for studying the neurophysiological basis of spatial hearing is shown in Fig. 1. In front of the preparation, five identical speakers (piezo tweeters; Motorola, bullet-type KSN1005) were positioned along a quarter circle at a distance of 90 cm. The speakers were elevated by 0°, 30°, 60°, 75°, and 90°; the latter exactly above the insect. The quarter circle with the speakers could be rotated by 180° (from 90° left to 90° right) in the azimuth around
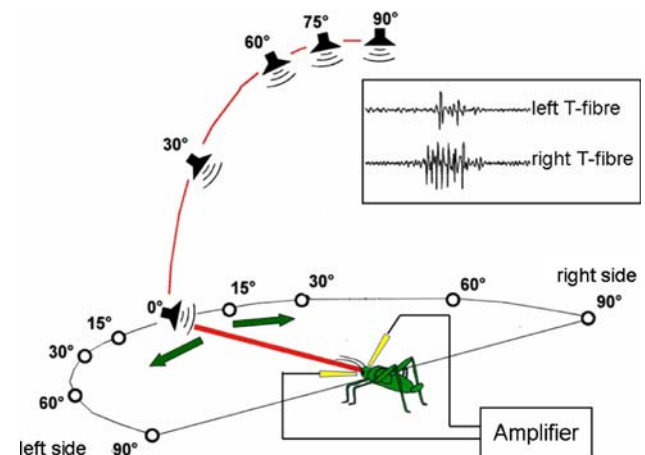

Fig. 1 Experimental set-up to study spatial directional hearing in *Leptophyes punctatissima.* The neurophysiological preparation with a bilateral extracellular recording of a pair of interneurons (T-fibre) is in the centre of an arrangement of five identical speakers positioned along a quarter circle at different elevations. The quarter circle with the speakers could be rotated by 180° (from 90° left to 90° right) in the azimuth around the centrally fixed preparation. When the preparation was oriented with its dorsal side upwards, the anterior dorsal sound field could be examined. Subsequently, after turning the preparation with its ventral side upwards, the spatial information was completed for the ventro-frontal hemisphere as well. The *inset* shows an example of a bilateral recording of the T-fibres in response to a mimic of the female reply (duration 1 ms) at a stimulus angle of 90° right in the horizontal plane. Note the bilateral difference in both response strength and latency. For further explanations see text

the centrally fixed preparation; thus stimulus angles in the azimuth could be changed in steps of 30°, and in front of the preparation in steps of 15°. With this arrangement, the anterior dorsal sound field could be examined, when the insect preparation was oriented with its dorsal side upwards. After completion of measurements in this dorso-frontal hemisphere, the preparation was turned with its ventral side upwards, and the spatial information was completed for the ventro-frontal hemisphere as well. The experimental set-up was positioned in an acoustically isolated room with the walls covered with plastic foam (thickness 5 cm) so that the echoes of the acoustic signal at 40 kHz as well as background noise were well below detection threshold of the insect.

Neurophysiology

We studied spatial hearing by recording the action potential activity of a pair of identified auditory interneurons, originally described as T-fibres by Suga and Katsuki (1961). The neuron originates in the prothoracic ganglion and exhibits an axon ascending to the brain as well as an axon descending to the abdominal ganglia (for a complete staining of the homologous neuron in another bushcricket, see Stumpner 1999). Dendritic branches are located within the auditory neuropile in the prothoracic ganglion, where they overlap, and are connected with, terminal branches of most receptors fibres, which are organized tonotopically (Römer 1983; Römer et al. 1988; Ebendt et al. 1994; Stumpner 1996). The T-fibre thus responds to a wide range of frequencies from about 5 kHz to more than 50 kHz (Rheinlaender and Römer 1980; Stumpner 1999; Schul and Sheridan 2006; Faure and Hoy 2000a). In addition to the ipsilateral excitatory input, the neuron receives inhibitory input from the contralateral ear, which makes it an ideal candidate to study peripheral and central nervous mechanisms of directional hearing. Furthermore, its large axon running laterally within the anterior connectives allow to record action potentials with extracellular hook electrodes (see below).

Details of the preparation, and methods for bilateral recording action potential activity of the pair of T-fibres, have been described elsewhere (Rheinlaender and Römer 1980). In short, the insect was first anaesthetized with CO_2, the hind and middle legs removed and the preparation fixed ventral side up with sticky wax on a small mounting platform. The platform was 2 mm wide and 0.5 mm thick, so that it was unlikely that its presence in the sound field contributed significantly to the observed directionality (see below). The tarsi of the forelegs were fixed to thin wires (diameter 0.5 mm) to achieve a natural leg position. The cervical cuticula was removed and the neck-connectives were placed onto the paired hook-electrodes and cut anteriorly next to the suboesophageal ganglion. After placement

of the hook electrodes the operating hole was sealed with petroleum jelly to prevent desiccation of the nervous tissue. The mounting with the preparation extended to a 10 cm long tube which could be clamped at the centre of the experimental set-up. Responses of both T-fibres were amplified using custom-made amplifiers, visualized through an oscilloscope (Agilend; 54616B Oscilloscope) and recorded on a DAT-recorder (TEAC; RD-120TE DAT DATA Recorder) for off-line analysis.

Acoustic stimulation

In Leptophyes pair formation is achieved by duetting, as the male song elicits an acoustic reply in the female, to which the male then responds by phonotaxis with very high probability (Hartley and Robinson 1976; Robinson 1980; Robinson et al. 1986; Zimmermann et al. 1989). We mimicked the female reply by triggering a short click of 1 ms duration at a frequency of 40 kHz from a sound generator (Fa. Burchard, akustischer Stimulator II). The signal was broadcast via one of the five speakers in front of the preparation. The sound pressure level (SPL) of the signal at the position of the preparation was calibrated with a sound level meter (Bruel &Kaer, type 2209) and a 1/4" microphone (type 4135) and is given in dB SPL (re 20 μPa; peak reading).

We performed two kinds of experiments: In a first series with a total of 10 animals the peripheral directionality in both azimuth and elevation was analysed in monaural preparations. In these experiments, the contralateral leg nerve (containing axons of auditory receptor fibres) was cut so that the ipsilateral T-fibre received only input from the remaining ipsilateral ear. By determining the threshold of the neuron for various positions of the sound source, the peripheral directionality of the system could be studied in azimuth and elevation. Threshold was defined as the sound pressure level which elicited one or more action potentials in the T-fibre in at least 8 out of 10 stimulus presentations. Stimulus duration was 1 ms; stimulus interval was 3 s.

The goal of the second series of experiments was to obtain spatial information coded in the activity of central neurons. For this purpose we performed experiments with bilaterally intact animals, by recording the responses of both, left and right T-fibres simultaneously, and varying the location of the sound source within the complete anterior sound field of the preparation. For each stimulus angle a series of 10 stimuli were delivered at a rate of one stimulus per 3 s. This stimulus rate is identical to the song rate of a male performing phonotaxis (Zimmermann et al. 1989; Rheinlaender et al. 2007). Furthermore, the T-fibre exhibits some degree of habituation in response to repetitive stimuli (McKay 1969; Faure and Hoy 2000b; Schul and Sheridan 2006), which is strongly reduced when using stimulus intervals based on the natural song rate of males. For each

stimulus angle 12–15 stimuli were presented, and the last 10 responses were later used for analysis, in order to prevent any effect due to habituation. Stimuli were broadcast at a sound pressure level of 60 dB SPL. Since the threshold of the neuron with ipsilateral stimulation is 37 dB SPL, this intensity is about 20 dB above the threshold. The analysis of bilateral discharge and latency differences was performed using the software Spike2 (Cambridge Electronic Design; England).

Results

Peripheral directionality

In order to investigate the peripheral directionality in the complete anterior sound field of the insect, the threshold of the T-fibre was measured, and the location of the sound source varied in azimuth and elevation. Figure 2a shows the
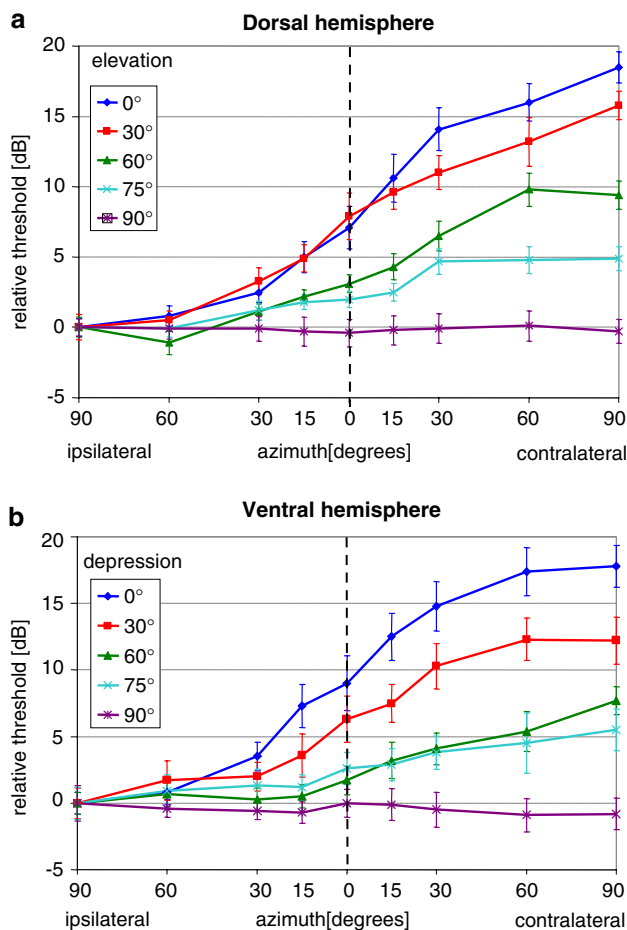

Fig. 2 Directionality of the ear of *L. punctatissima* at different elevations of the speaker in the dorsal (**a**) and ventral (**b**) hemisphere. For each elevation, thresholds are plotted relative the ipsilateral threshold at that elevation, using the discharge of a monaural T-fibre preparation (means ± SEM; $N = 10$)

results for the dorsal sound field. When the threshold of the monaural T-fibre preparation with ipsilateral stimulation is taken as a reference, and the angle of sound incidence was varied in the horizontal plane from 90° ipsilateral to 90° contralateral, there was a mean total shift in threshold increase of 18.5 dB. With more elevated sources, the total ipsi–contralateral difference decreased to 15.8 dB (30° elevation), 9.4 dB (60° elevation), and 4.9 dB (75° elevation), respectively (mean values of 10 preparations). With the source elevated by 90° (exactly above the preparation), there was of course no shift in threshold to be expected, because the speaker only rotates around its central axis, and indeed there was no threshold shift observed.

Identical measurements were performed in the ventral, anterior sound field; the results are shown in Fig. 2b, describing the ventral acoustic hemisphere of the preparation. Similar to the situation in the dorsal sound field, the threshold differences between ipsi- and contralateral stimulation decreased with increasing depression of the sound source from 17.8 dB in the azimuth to 12.2 dB (30° depression), 7.7 dB (60° depression) to 5.5 dB (75° depression; mean values of 10 preparations).

Interaural intensity differences (IIDs)

By assuming bilateral symmetry, the interaural intensity differences (IIDs) for each spatial location of the sound source can be calculated. The result of this calculation is shown in Fig. 3a, b for the dorsal and ventral acoustic hemispheres, respectively. From these data we can infer the amount of directional information as a result of the peripheral directionality for any spatial position of a source within the anterior sound field. There is both a decrease of IIDs with increasing elevation or a depression of a source and when the source is moved from a lateral position to the longitudinal body axis. For example, if the source is depressed by 60° and the insects maintains an azimuthal angle of 60° to the target, the peripheral directionality provides an IID of 5 dB to the central nervous system. Even when stimulated from a position 90° lateral, maximal IIDs of 7.5–8 dB are produced, and with smaller stimulus angles of 15° the resulting IID is 2.5 dB (see Fig. 3b).

Bilateral discharge differences in the CNS

An orienting insect does not rely on IIDs as a sensory cue. Instead, the observed peripheral directionality results in discharges of afferents and directionally sensitive interneurons, which are interpreted by the CNS for making correct decisions in phonotactic movements. We therefore recorded the discharge of the pair of directionally sensitive T-fibres simultaneously, while stimulating the preparation from different directions within the anterior sound field.
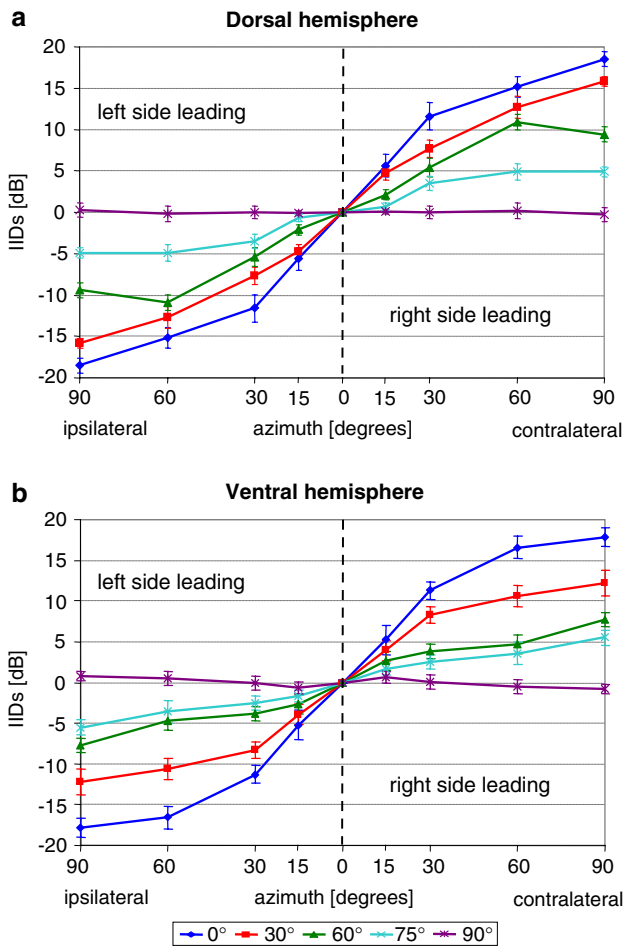
Fig. 3 Interaural intensity differences in the auditory system of *L. punctatissima* in the dorsal (**a**) and ventral (**b**) hemisphere under the assumption of a symmetrical hearing system. Note the decrease of IIDs with increasing speaker elevation or depression for any angular position in the azimuth. For further explanations see text

Fig. 4 Directionality of the discharges of a pair of T-fibres in the azimuth in response to the acoustic female reply (mean ± SD; $N = 10$; data for one preparation). Significant discharge differences are achieved with stimulus angles of 30° or more. Note the strong discharge of the T-fibre in response to a stimulus of 1 ms duration, mimicking the female reply

The directionality of discharges for changes in speaker location in the azimuth is shown in Fig. 4 for one preparation. Both neurons show maximal and minimal discharges with ipsilateral and contralateral stimulations, respectively. Response strength decreased systematically with speaker positions to the contralateral side. In the preparation shown in Fig. 4, there was a slight asymmetry in the frontal zone, because almost identical discharges are elicited at stimulus angles between 15° left and frontal stimulation at zero degrees. This general pattern of directionality is a result of contralateral inhibition and has been reported for the same homologous pair of interneurons in other katydids as well (e.g. Rheinlaender and Römer 1980). Remarkably for Leptophyes, however, the neurons respond with ipsilateral stimulation with 6–7 action potentials to a stimulus of only 1 ms.

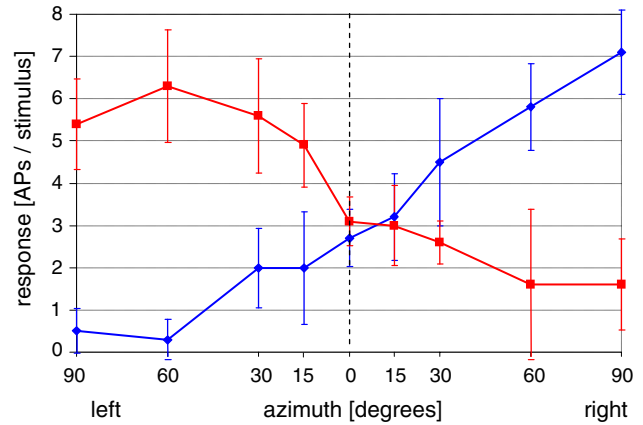The results of 10 bilateral experiments, performed at different elevation angles in the dorsal hemisphere, are shown in Fig. 5a. In the dorsal hemisphere, and with the horizontal speaker position, the calculated bilateral discharge differences decreased systematically from more than 4 APs ipsilaterally to exactly zero at frontal stimulation, and the same differences developed on the opposite side with the opposite neuron leading. The strongest gradient was observed with an azimuthal shift in speaker position in the frontal zone of ±30°. When the speaker was elevated by 30°, the pair of T-fibres exhibited almost identical gradients in binaural spike difference, whereas at higher elevation angles of 60° and 75° the absolute differences decreased to 2.5 and 1 AP/stimulus with lateral stimulation, and the gradients in the frontal zone of the preparation decreased as well. A similar pattern of directionality was observed in the ventral anterior hemisphere (not shown in Fig. 5).

In addition to bilateral differences in spike discharges, the latency difference may represent a binaural cue for directional coding as well (Mörchen et al. 1978; Rheinlaender and Mörchen 1979; Samson and Pollack 2002). We therefore analysed bilateral latency differences in the pair of T-fibres at different positions of the sound source in azimuth and elevation (Fig. 5b). Latency differences amount to more than 6 ms at lateral stimulus angles in the azimuth, and with a source elevation of 0° and 30°. The latency differences decrease to 3 ms at 60° and only about 1 ms at 75° elevation. A similar dependency was found in the ventral hemisphere with depressed sound source positions. Thus, in addition to bilateral discharge differences, latency differences between 1 and 6 ms also represent cues for spatial orientation. The values of bilateral discharge differences as well as latency differences for a lateral sound source are summarized in Table 1.
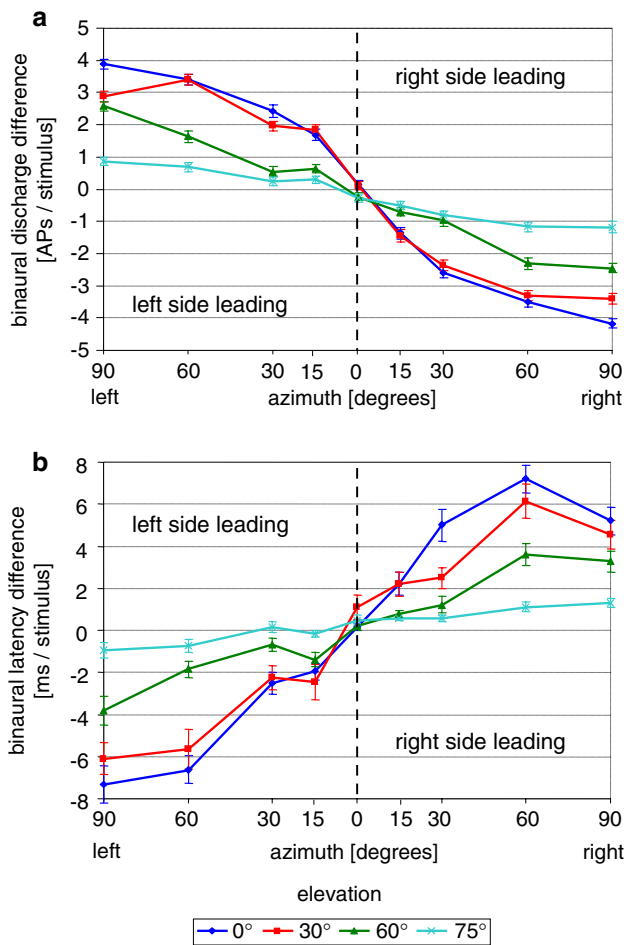
Fig. 5 Directionality of the discharges in 10 preparations of a pair of T-fibres in the dorsal hemisphere, in response to the acoustic female reply. **a** Bilateral discharge difference; **b** bilateral latency difference; *negative values* refer to a situation where the right side is leading; (mean ± SEM; *N* = 10)

Table 1 Summary of bilateral discharge differences and bilateral latency differences in the pair of T-fibres at different sound source elevations, and an azimuthal angle of 90° (means ± SEM)

Elevation [degrees]	Bilateral discharge differences [APs/stimulus ±SEM]	Bilateral latency differences [ms/stimulus]
0	4.04 ± (0.10)	6.35 ± (0.55) N = 129
30	3.15 ± (0.11)	5.29 ± (0.52) N = 139
60	2.52 ± (0.11)	3.55 ± (0.42) N = 164
75	1.03 ± (0.11)	1.11 ± (0.21) N = 197

Discussion

In two previous papers we have examined the spatial orientation behaviour of male *L. punctatissima*. When males oriented in a three-dimensional artificial grid system towards a horizontal loudspeaker, or those elevated or depressed by 45°, they reached each of the three loudspeaker positions with only little deviation from the shortest possible path (Rheinlaender et al 2007). Quantitative measures of the accuracy of orientation were not different between the three source positions. In addition, the orientation of males was also examined on a walking compensator, where calling males received a synthetic female reply from speaker positions varying in elevation from 0° (horizontal) to 90° (exactly from above the insect; Ofner et al. 2007). Due to the automatic compensation of the insects' walk the elevation angle was kept constant throughout the complete phonotactic sequence. The hypothesis underlying these experiments was that with increasing speaker elevation the binaural directional cues available for the male should decrease up to a point where spatial orientation is either not possible or severely impaired. In the present paper we examined the directional cues underlying the spatial orientation of males in two ways: first, we measured the directionality of the bushcricket ear in azimuth and elevation, and second, the central nervous representation of spatial cues was measured using the bilateral discharges of a homologous pair of directionally sensitive interneurons in response to a female reply broadcast from locations within the frontal hemisphere.

Peripheral directionality

The peripheral directionality of the ear of *L. punctatissima* at the females' call carrier frequency of 40 kHz is rather high in the azimuth, with a maximal ipsi–contralateral difference of about 18 dB. This value is within the range of variation found in a combined behavioural and neurophysiological study on females of the same species (13–19 dB; Rheinlaender et al. 1986). With increasing elevation or depression of the sound source, these values decrease in a more or less systematic fashion (Fig. 3), so that the gradient of IIDs could be used by the male for his directional decisions during phonotaxis. If, for example, a male approaches an elevated female from some distance, the perceived elevation angle might be 30°, and by regularly meandering with right and left turns of e.g. 30° in the horizontal plane, this would create maximal IIDs of about 8 dB. If the male continues to move in the horizontal plane, the perceived elevation angle will increase with decreasing distance, and the same right–left turns of 30° will create smaller IIDs of 4 dB at a source elevation angle of 75°. Only if the male follows in his approach the source elevation by climbing up, he will be able to operate with the largest possible IIDs for a given turn angle. The same would be true with depressed sound sources.

However, there is an obvious ambiguity in the peripheral directional cues available for a receiver: a depressed sound

source of e.g. 30° may result in almost the same IID of 8 dB with a turn angle of 30° as does an elevated sound source (7.5 dB; Fig. 3). How can the male solve this problem? There are two possible solutions: for example, if the source is elevated by 30°, and the male falsely decides to climb down rather than up, the next female responses will arrive from a much more elevated angle, so that the IIDs may rapidly decline to almost zero. Assuming that the male has some kind of short-term memory by comparing the current sensory information with that of the previous one (or more previous ones), he would be able to correct his path and climb up towards the source the next time.

The second possibility is to avoid walking into the wrong direction in the case of ambiguous information, by performing some kind of acoustic scanning beforehand. Indeed, we had observed a tilting behaviour especially at the beginning of a phonotactic trial. In general, the male tilted its head and thorax downward, and often simultaneously performed a certain yaw-angle and rolled its body axis to one or the other side (Ofner et al. 2007). We therefore hypothesized that, altering these orientations of the body relative to the sound source leads to self-induced changes of binaural differences. Figure 6 illustrates this effect for the tilting (head down) component, with a sound source fixed in the horizontal plane of the male (based on results presented in Fig. 3). The colour-coded IIDs give a visual impression for the interaural changes perceived by the orienting male. The strongest changes in IIDs occur when the male turns right or left and does not tilt. The same is true when the insect tilts its body by only 30°. However, there is a substantial loss in directionality when the tilting angle is between 60° and 90°. The opposite would occur

with a depressed sound source: here, tilting would bring the source closer to the males' horizontal plane, and thus would create large IIDs. Our own preliminary observations confirmed that males performed such large tilting angles during a forward bending of their head and thorax between 60° and 90°, thus making use of the strongest gradient in the changes of IIDs which occur within this spatial position. Thus, by tilting the male could actively alter its perceived binaural cues, much in the same way as a mammal could obtain spatial cues by moving its pinnae (Searle et al. 1975). By comparing successive tilting manoeuvers (and the resulting changes in binaural cues), the insect could obtain at least an effective estimate of whether the source is above or below, or left or right. This mechanism would enable the insect to correctly orient without an initial incorrect movement into the wrong direction. Again, this mechanism requires some kind of short-term memory in order to compare the result of successive tilting postures or localization positions. We currently perform a detailed analysis of the described acoustic scanning behaviour and its underlying physiological mechanisms.

Central nervous spatial cues

Although the peripheral auditory system of *L. punctatissima* provides excellent cues for spatial hearing, the accuracy of phonotaxis in the grid system (Rheinlaender et al. 2007) or on the walking belt (Ofner et al. 2007) is nevertheless remarkable, given the fact that the ultimate cues for the insect are bilateral differences in response strength and/or response latency in the afferent auditory pathway (Pollack 2000 for review). In contrast to other acoustic insects which either call continuously or produce long duration song elements, a male *L. punctatissima* must orient in response to an extremely short female reply of less than 1 ms duration. Auditory receptors in the ear respond with no more than one action potential to such a short stimulus, irrespective of the sound pressure level (Hardt 1988). As a consequence, there is neither a dynamic intensity response function of a single receptor, nor a graded information available in the single receptor to changing stimulus angles. Processing this information is only possible using a population code either via the number of fibres activated, because they differ in their absolute sensitivity and/or tuning (Hardt 1988), or via changes in the degree of synchronization, which could influence the responses of interneurons. Oshinsky and Hoy (2002) have provided evidence, that such a population code, based on differential recruitment of receptor cells, plays a role in directional hearing of a parasitoid fly. To study bilateral discharge differences available for the brain we recorded the activity of a pair of interneurons simultaneously in response to such short stimuli broadcast within the dorsal and ventral anterior hemisphere of the insect.
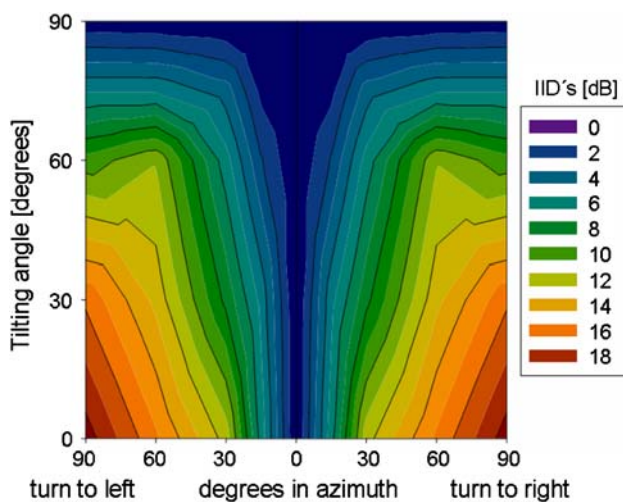
Fig. 6 Colour-coded interaural intensity differences in the peripheral system of *L. punctatissima* created by a male turning to right or left at different tilting (head and thorax down) angles, with the sound source at a frontal position in the horizontal plane. IIDs are based on data presented in Fig. 3

Our choice was the so-called T-fibre, and several properties of the cell are consistent with the assumption that it is involved in the ultrasound-triggered startle response during flight, in particular the large axon diameter and presumed monosynaptic connection to receptor fibres (Römer et al. 1988), favouring a fast behavioural response towards predators. Further, the physiology of the cell appears to be biased towards responses to bat-like sound rather than conspecific mating calls (Faure and Hoy 2000a, b; Schul and Sheridan 2006). Although we do not argue here that the T-fibre in Leptophyes is causally involved in guiding phonotaxis towards females, one may speculate about a possible functional switch of this prominent cell in this Tettigoniid species, which is flightless and therefore the presumed function in bat avoidance during flight is lost.

In response to the short female reply, the T-fibre produces a rather strong response of up to seven action potentials/stimulus on average with ipsilateral stimulation. More importantly, the discharge differences of the pair of T-fibres exhibit a systematic shift with sound direction in the azimuth, and decrease with both the elevation and depression of the sound source (Fig. 5a). In this respect, these data corroborate the peripheral data described before. Remarkably, with an elevation of the source of 60°, where most males oriented quite well on the walking belt (Ofner et al. 2007), discharge differences in the pair of T-fibres amount to two action potentials at a lateral stimulus angle of 60° and only one action potential with a lateral stimulus angle of 30°. Some males on the walking belt still performed accurate phonotaxis when the source was elevated by 75°, which would suggest that they had to base their orientation on the minute discharge difference of just one AP. However, this is not necessarily the case, because in two individual preparations these binaural discharge differences at an elevation angle of 75° have been significantly higher than on average, which might well represent the neuronal basis for the observed differences in behavioural performance at this critical elevation angle. Based on simultaneously recorded responses of the pair of T-fibres in 10 preparations we also performed an analysis of the accuracy of lateralisation in the dorsal hemisphere, if a male based his decisions on such a comparison (Fig. 7). We analysed the data using either bilateral discharge differences alone or in combination with bilateral latency differences (Fig. 7a, b). A bilateral coding was considered correct if the T-fibre ipsilateral to the stimulus fired at least one action potential more per stimulus than its counterpart, or fired at least 1 ms earlier. For the symmetrical, frontal situation coding was considered correct when the discharge in both neurons was the same, or latency differences were less than 1 ms. In the combined analysis for correct coding both conditions should be met. The results for the bilateral discharge differences alone (Fig. 7a) indicate a sharp decrease in correct responses for

frontal stimuli, starting at stimulus angles of ±30° in the azimuth, and for elevation angles of 0° and 30°, whereas for more lateral stimulus angles correct responses are above 90%. For more elevated sound sources of 60° and 75°, however, correct responses in the lateral dorsal hemisphere range between 60 to 90%, and between 40 to 60% at stimulus angles of ±30° in the azimuth. Including bilateral latency differences as an additional criterion for correct responses (Fig. 7b) reduces the percentage of correct responses, but shows a similar tendency of decreasing correct responses with increasing elevation angle.

The neurophysiological data are also consistent with the observed gradual decrease in the correlation between stimulus angle and turn angle with increasing source elevation on the walking belt (Ofner et al. 2007). Although in the azimuth and at an elevation of 30° the binaural differences indicate a reliable coding of the stimulus angle, the steepness declines rapidly with more elevated sound sources. Thus, smaller stimulus angles provide only unreliable or erroneous information about the location of the source, and, as a consequence, the insect would have to make larger turns in order to track the target.
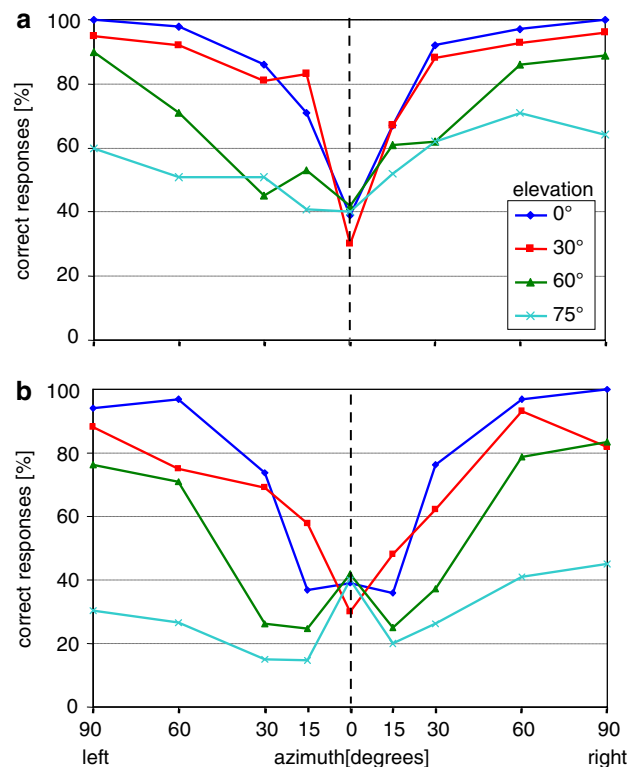

Fig. 7 Percentage of correct lateralisation coding based on simultaneously recorded responses of the pair of T-fibres for sound sources in the dorsal hemisphere. In **a** only bilateral discharge differences were used for analysis, whereas in **b** bilateral discharge and latency differences were used. For further information see text

References

Blauert J (1983) Spatial hearing. The psychophysics of human sound localization, 2nd edn. MIT Press, Cambridge

Butler RA, Belendiuk K (1977) Spectral cues utilized in the localization of sound in the medial sagittal plane. J Acoust Soc Am 61:1264–1269

Ebendt R, Friedel J, Kalmring K (1994) Central projection of auditory receptors in the prothoracic ganglion of the bushcricket *Psorodonotus illyricus* (Tettigoniidae): computer-aided analysis of the end branching pattern. J Neurobiol 25:35–49

Faure PA, Hoy RR (2000a) Neuroethology of the katydid T-Cell. I. Tuning and responses to pure tones. J Exp Biol 203:3225–3242

Faure PA, Hoy R (2000b) Neuroethology of the katydid T-Cell. II. Responses to acoustic playback of conspecific and predatory signals. J Exp Biol 203:3243–3254

Fuzessery ZM (1996) Monaural and binaural spectral cues created by the external ears of the pallid bat. Hear Res 95:1–17

Gerhardt HC, Huber F (2002) Acoustic communication in insects and anurans: common problems and diverse solutions. The University of Chicago Press, Chicago

Gerhardt HC, Rheinlaender J (1982) Localization of an elevated sound source by the green tree frog. Science 217:663–664

Hardt M (1988) Zur Phonotaxis von Laubheuschrecken: Eine vergleichende verhaltensphysiologische und neurophysiologisch-anatomische Untersuchung. PhD Thesis, Ruhr-University Bochum

Hartley JC, Robinson DJ (1976) Acoustic behaviour of both sexes of the speckled bush cricket *Leptophyes punctatissima*. Physiol Entomol 1:21–25

Hoy RR, Robert D (1996) Tympanal hearing in insects. Ann Rev Entomol 41:433–450

Knudsen EI, Konishi M (1979) Mechanism of sound localization in the barn owl. J Comp Physiol 133:13–21

Konishi M (2003) Coding of auditory space. Annu Rev Neurosci 26:31–55

McKay JM (1969) The auditory system of *Homorocoryphus* (Tettigonioidea, Orthoptera). J Exp Biol 51:787–802

Mörchen A, Rheinlaender J, Schwartzkopff J (1978) Latency shift in insect auditory nerve fibers. Naturwiss 65:656

Müller P, Robert D (2001) A shot in the dark. The silent quest of a free-flying phonotactic fly. J Exp Biol 204:1039–1052

Ofner E, Rheinlaender J, Römer H (2007) Spatial orientation in the bushcricket *Leptophyes punctatissima* (Phaneropterinae; Orthoptera): II. Phonotaxis to elevated sound sources on a walking compensator. J Comp Physiol A 193:321–330

Oshinsky ML, Hoy RR (2002) Physiology of the auditory afferents in an acoustic parasitoid fly. J Neurosci 22:7254–7263

Payne RS, Roeder KD, Wallmann J (1966) Directional sensitivity of the ears of noctuid moths. J Exp Biol 44:17–31

Popper AN, Fay RR (2005) Sound source localization. Springer handbook of auditory research, vol 25. Springer, Heidelberg

Pollack G (2000) Who, what, where? Recognition and localization of acoustic signals by insects. Curr Opin Neurobiol 10:763–767

Rheinlaender J, Hardt M, Robinson DJ (1986) The directional sensitivity of a bush cricket ear: a behavioural and neurophysiological study of *Leptophyes punctatissima*. Physiol Entomol 11:309–316

Rheinlaender J, Mörchen A (1979) "Time-intensity trading" in locust auditory interneurones. Nature (London) 281:672–674

Rheinlaender J, Römer H (1980) Bilateral coding of sound direction in the CNS of the bushcricket *Tettigonia viridissima L.* (Othoptera, Tettigoniidae). J Comp Physiol A 140:101–111

Rheinlaender J, Hartbauer M, Römer H (2007) Spatial orientation in the bushcricket *Leptophyes punctatissima* (Phaneropterinae; Orthoptera): I. Phonotaxis to elevated and depressed sound sources. J Comp Physiol A 193:313–320

Robinson DJ (1980) Acoustic communication between the sexes of the bush cricket *Leptophyes punctatissima*. Physiol Entomol 5:183–189

Robinson DJ, Rheinlaender J, Hartley JC (1986) Temporal parameters of male–female sound communication in *Leptophyes punctatissima*. Physiol Entomol 11:317–323

Römer H (1983) Tonotopic organization of the auditory neuropile in the bushcricket *Tettigonia viridissima*. Nature 306:60–62

Römer H, Marquart V, Hardt M (1988) Organization of a sensory neuropile in the auditory pathway of two groups of Orthoptera. J Comp Neurol 275:201–215

Samson AH, Pollack GS (2002) Encoding of sound localization cues by an identified auditory interneuron: effects of stimulus temporal pattern. J Neurophysiol 88:2322–2328

Schul J, Sheridan RA (2006) Auditory stream segregation in an insect. Neuroscience 138:1–4

Searle CL, Braida LD, Cuddy DR, Davis MF (1975) Binaural pinna disparity: another auditory localization cue. J Acoust Soc Am 57:448–455

Stumpner A (1996) Tonotopic organization of the hearing organ in a bushcricket—physiological characterization and complete staining of auditory receptor cells. Naturwiss 83:81–84

Stumpner A (1999) An interneurone of unusual morphology is tuned to the female song frequency in the bushcricket *Ancistrura nigrovittata* (Orthoptera, Phaneropterinae). J Exp Biol 202:2071–2081

Suga N, Katsuki Y (1961) Central mechanisms of hearing in insects. J Exp Biol 38:545–558

Wotton JM, Simmons JA (2000) Spectral cues and perception of the vertical position of targets by the big brown bat, *Eptesicus fuscus*. J Acoust Soc Am 107(2):1034–1041

Wyttenbach RA, Hoy RR (1997) Spatial acuity of ultrasound hearing in flying crickets. J Exp Biol 200:1999–2006

Zimmermann U, Rheinlaender J, Robinson DJ (1989) Cues for male phonotaxis in the duetting bushcricket *Leptophyes punctatissima*. J Comp Physiol A 164:621–628