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Auditory lateralization in bushcrickets: a new dichotic paradigm

Abstract Pair formation in the bushcricket *Gampsocleis gratiosa* is achieved through acoustic signalling by the male and phonotactic approaches of the female towards the calling song. On a walking belt in the free sound field, females tracked the position of the speaker broadcasting the male calling song with a remarkable precision, deviating by no more than 10 cm in either direction from the ideal course. Starting with stimulus angles of 6–10° the females significantly turned to the correct side, and with stimulus angles greater than 25° no incorrect turns were made. Using neurophysiological data on the directionality of the ear we calculated that with such stimulus angles the available binaural intensity difference is in the order of 1–2 dB. We developed a dichotic ear stimulation device for freely moving females with a cross-talk barrier of about 50 dB, which allowed to precisely apply small binaural intensity differences. In such a dichotic stimulation paradigm, females on average turned to the tronger stimulated side starting with a 1 dB difference between both ears. The significance of such a reliable lateralization behaviour with small interaural intensity differences for phonotactic behaviour under natural conditions is discussed.

Keywords *Gampsocleis gratiosa* · Bushcricket · Directional hearing · Dichotic stimulation · Binaural cues

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Abbreviations IID: Interaural intensity difference · SPL: Sound pressure level

Introduction

Regen (1913) described the phonotactic approach of a female cricket towards a telephone speaker broadcasting a male calling song. His pioneering work initiated a number of studies on the sound communication behaviour in crickets, grasshoppers and bushcrickets. Mate finding in these insects involves both the recognition of sound signals, as well as the localisation of, and movement to, the source. Results on the acoustic behaviour of males and females, and the underlying biophysical and neurophysiological mechanisms have been reviewed extensively (Ewing 1989; Huber 1989, 1990; Bailey and Rentz 1990; Bailey 1991; von Helversen 1997; Michelsen 1998; Pollack 1998, 2000; Gerhardt and Huber 2002). Either the female moves phonotactically to the calling, stationary male, or she performs a sound duet with the male which then moves to the female. Irrespective of the sex performing phonotaxis, the results of these approaches indicate that the phonotactic path differs in general from a straight line; instead, it resembles a meandering zigzag course based on successive course corrections. In grasshoppers, for example, the phonotactic approach is characterized by a series of stereotyped right–left discriminations (von Helversen and von Helversen 1983; Römer and Rheinlaender 1989; von Helversen 1997). Other insects are able to scale their turning responses according to the position of the sound source. This is true for crickets in arena trials (Murphey and Zaretzky 1972; Bailey and Thompson 1977; Latimer and Lewis 1986), or on a locomotor compensator (Schmitz et al. 1982; Weber et al. 1981). The speed of turns in response to the calling song was also found to be positively correlated with the angular deviation of the animal from the source (Schmitz et al. 1982; Stabel et al. 1989). In the escape response of flying crickets to

ultrasound the steering movements in tethered flight are larger with more lateral speaker positions (Pollack and Plourde 1982). A linear relationship between angular deviation and the turning response was also found in bushcrickets (Hardt 1988; Rheinlaender and Römer 1990). Irrespective of the phonotaxis, based simply on a right–left discrimination (lateralization) or an angular-dependent turning behaviour the minimum angle for correct auditory side discrimination in grasshoppers and crickets appears to be in the order of 10–15° (Rheinlaender and Blätgen 1982; von Helversen 1997).

Although it has been demonstrated recently, that female lesser wax moth (*Achroia grisella*) can orient to a sound source when binaural acoustic differences are not available, by using sequential comparison of the sound field (Greenfield et al. 2002); it is clear that insects usually base their phonotactic approaches on a binaural comparison. Except for situations of symmetrical stimulation (source exactly in front, behind or above the insect) interaural intensity cues are available for the orientation to the source. One source of evidence for the minimum binaural cues involved for orientation stems from female choice experiments with two sound sources presented from opposite directions (partly summarized by Forrest 1994 and Römer et al. 1998; see also Gerhardt and Huber 2002, and literature therein). Depending on species, insects prefer one source over the other when the difference is 1–3 dB. However, with respect to orientation towards a single sound source, there is a general lack of information about the minimum interaural cues involved. The most obvious reason is that body size, and hence interaural disparities, is minute in insects, which impairs the application of headphones or any kind of independent ear stimulation device. Moreover, in crickets and grasshoppers the two hearing organs are acoustically coupled via tracheal structures (review Bailey 1991; Michelsen 1998) which is a further disadvantage for dichotic (independent ear stimulation) experiments, because the extent to which one ear receives a supra-threshold sound signal from stimulation of the other ear remains largely unknown. Our present knowledge is, therefore, only based on one estimate in crickets (Rheinlaender 1984) and on a tentative behavioural approach in a grasshopper (von Helversen and Rheinlaender 1988). Both studies indicate that the insects' auditory system can make use of interaural intensity differences (IIDs) in the order of 1–2 dB. It should be noted that such differences result mainly from the complex biophysical receiver characteristics of the peripheral ear structures (see Michelsen 1998 for review).

In this paper we present results on the minimum binaural intensity difference for correct lateralization based on a new method of dichotic ear stimulation in a freely walking bushcricket. The stimulation paradigm provides a high cross-talk barrier between the two ears and enables the accurate determination of interaural intensity cues involved in directional hearing.

Materials and methods

Animals and stimulation

Dichotic ear stimulation in freely behaving insects requires the stimulation equipment to be as small as possible, such that the usual phonotactic movement is not altered. The development of miniature earphones for humans (Knowles subminiature transducers Type ED 2913, size 6.35×4.34×3.02 mm^3, weight 0.31 g) enabled the design of a dichotic stimulation paradigm in a freely moving bushcricket, the size of which is large enough to carry such a device without influencing its behaviour. This is the case for the Asian tettigoniid *Gampsocleis gratiosa*, a rather large bushcricket (body length 50–55 mm without ovipositor; width of pronotum 0.8–0.9 mm; weight 3.6–3.8 g), and thus appropriate for this study. In this species the spiracular openings of the acoustic trachea are so large, that sound conducting tubes can be applied to the acoustic trachea on each side (Fig. 1a). This is a prerequisite for bilateral independent acoustic stimulation in a freely moving female. A small plastic backpack as carrier of the two earphones was developed that could be attached to the pronotum of the behaving insect (Fig. 1a). The output of these earphones in the backpack was oriented downwards, each coupled to the ipsilateral acoustic spiracle via short, flexible plastic tubes. To avoid acoustic "leakage" and to achieve stable fixation during walking, the end of the plastic tubes was sealed into the spiracular openings with dental wax. Thus a direct coupling between each miniature speaker and the ear on each side was achieved. The weight of the two transducers including the plastic backpack and attached tubes was 0.772 g.

A further prerequisite for this approach is a match between the frequency characteristic of the earphone and the essential frequencies used by the animal for phonotaxis. The calling song spectrum of a male *G. gratiosa* calling song ranges from about 3 to 40 kHz with major peaks at about 3.5, 7 and 11 kHz (Shen and Tang 1991; Kalmring et al. 1995; Fig. 1b). By contrast, the frequency characteristic of the Knowles earphone system has a resonant frequency at about 2 kHz and declines sharply to higher frequencies, starting at about 16 kHz. Thus we compensated for this mismatch by digitally adjusting the amplitudes of the frequency bands at 3.5, 7 and 11 kHz by the same degree, so that they had the same relative amplitude as in the normal calling song when measuring the output of the transducer system with a probe microphone. Furthermore, we limited the frequency range of the signal to 15 kHz. Thus the effective frequency spectrum of the stimulus mimicked the spectrum of the natural calling song only up to about 15 kHz.

In order to calibrate and check for harmonic distortions in the plastic tube system we used a probe microphone type 4182 (frequency response up to 20 kHz, albeit reduced by 8 dB at 20 kHz with a tube of 25 mm
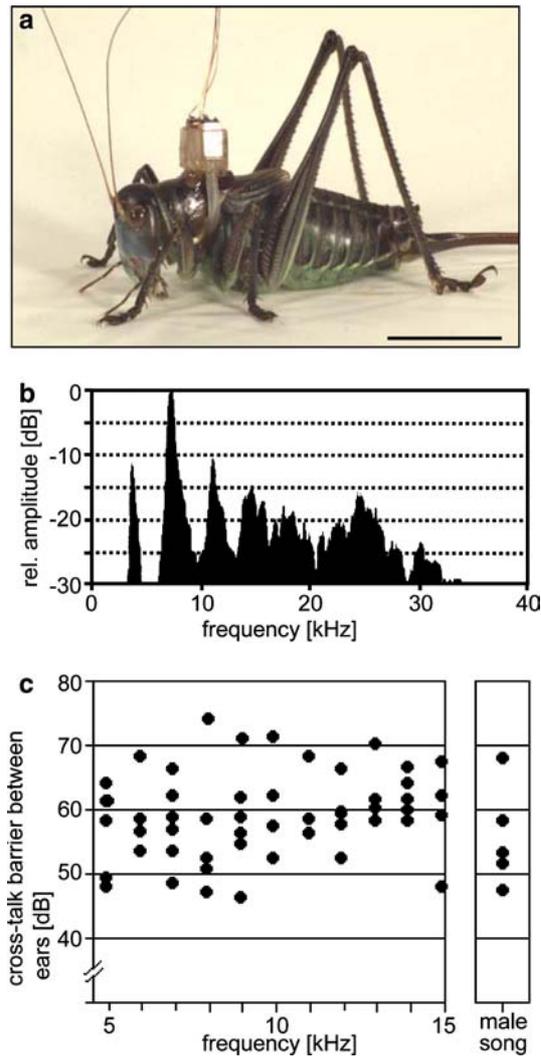
Fig. 1 a Female *Gampsocleis gratiosa* with a dichotic ear stimulation device. A backpack is attached to the dorsal pronotum, carrying two miniature speakers (size 6.35×4.34×3.02 mm³). Each speaker is connected via plastic tubes to the large spiracular opening of the acoustic trachea of the respective ipsilateral side, so that sound can be separately applied through the leg trachea to the inner side of each ear. *Scale bar* = 2 cm. **b** Spectrum of the calling song of a male *G. gratiosa*. For stimulation with the earphone system the amplitude of the spectrum was digitally adjusted to the frequency characteristic of the earphone, and limited to frequencies below 15 kHz. **c** Neurophysiologically determined cross-talk barrier between the two ears achieved with the dichotic stimulation shown in **a**, using the unilateral omega-cell preparation. The cross-talk barrier is given as the difference in the threshold SPL of the unilaterally activated omega-cell between ipsilateral and contralateral stimulation, with pure tones between 5 and 15 kHz, and also for the calling song. The contralateral ear was functionally eliminated by cutting the leg nerve carrying the axons of afferent auditory fibres

length). The high impedance of the small tip of the probe allows measurements in very small volumes of about 1 cm² , and although the applied spiracular tube system had a smaller volume, it was of the same order. With the various pure tones and stridulatory signals used in the study we did not find harmonic distortions even 45 dB below the maximum amplitude at higher frequencies,

which could have produced large errors when using the HF-sensitive omega-cell for the calibration procedure.

The calling song of a male *G. gratiosa* is a series of continuously repeated chirps (chirp duration of about 300 ms) at a rate of 2/s. The female tracks this signal in a continuous walk with only little deviations from the direct path (Shen 1985). In order to analyse the stimulus-related turning response we used an interrupted signal with three chirps in succession (chirp duration 300 ms; chirp interval 250 ms). This series of three chirps was repeated every 2 s, which still elicited a reliable phonotactic response and which enabled us to analyse the relationship between the presented sound signal and a correlated turning response. The stimulus used in all experiments was derived from a calling song of an isolated male, which was recorded in an anechoic room with a 1/4″ microphone (Bruel and Kjaer, Type 4163), preamplified through a sound level meter (Bruel and Kjaer, Type 2209). The signal was digitised on a custom-made AD/DA converter (12 bit; sampling rate 125 kHz) and broadcast through a wide-band amplifier (Burchard II). For the free-field stimulation experiments, either a song with the full frequency spectrum was used, or a high-pass filtered song with a cut-off frequency of 11 kHz was used (see Fig. 3). For the dichotic stimulation, only the song with a spectrum limited to 15 kHz was used.

A total of 45 female *G. gratiosa* was used both for neurophysiology and for behavioural phonotactic experiments. They were collected as larvae in field locations of the suburban region of Yixian County (Hebei Province, China) and airmailed to Bochum (Germany), where all experiments were performed. Animals were kept isolated in wooden boxes until the final moult at an L/D cycle of 12/12 h. They were fed on a combined plant–protein diet consisting of leek and pellets of dog and fish food.

Behavioural experiments

In a first set of experiments unrestrained females were tested on a walking belt to analyse the accuracy of the phonotactic path. Females were tested with chirps of the natural calling song at intensities of 65 dB SPL (re 20 µPa). In a series of preliminary experiments, the behavioural threshold for phonotaxis was tested with the full spectrum of the calling song, which varied in 10 females from 39 to 46 dB SPL (mean 42 dB SPL). Thus, the sound pressure level used in the free-field experiments was about 20–25 dB above the behavioural threshold. In a separate series of experiments, the speed of phonotaxis was compared at three different intensities (45, 65 and 80 dB SPL) in five females. Females were positioned at the centre of a horizontal platform provided by the walking belt (width 50 cm, length 80 cm). In front of the system, and along its longitudinal axis, a speaker (AUDAX TW8 Special) was positioned at a distance of 1 m relative to the centre of the walking belt.

Any forward or backward movement of the animal was compensated by the operator by turning the walking belt mechanically in the opposite direction. This maintained the walking insect at a constant distance (and thus constant intensity) to a speaker. Thus, in contrast to the Kramer-treadmill (Kramer 1976; Weber et al. 1981; Schmitz et al. 1982), only forward or backward movements, but no lateral movements are compensated. Although the walking belt was operated manually, even the faster speed of a female allowed to rather precisely control its position by the operator, so that the ''jitter'' in the anterior/posterior direction (distance) was not more than 5 cm. Thus, in terms of SPL the effect was less than 0.5 dB and negligible.

The phonotactic paths of the animals were recorded by a residual light-sensitive video system (camera JVC, GS 4600E) mounted 1.5 m above the centre of the walking belt. In addition, the signal of a LED indicating the onset and duration of the sound signal was displayed in the video frame. Thus the orientation and walking path of the animal could later be analysed frame by frame (recorder JVC, HR 2200E) with particular focus on the relationship between stimulus angle (speaker axis relative to the longitudinal body axis) and turning angle (defined as the difference in the longitudinal body axis before and 200 ms after the termination of the acoustic stimulus). The accuracy of the analysis was $\pm 1°$. The phonotactic path of females was reconstructed from a single frame video analysis, by monitoring the position of the pronotum of females after each stimulus presentation on the grid of the walking belt. Experiments were performed in an anechoic room ($3 \times 5 \times 3$ m^3, Grünzweig and Hartmann), illuminated by a 15 W red light bulb, just sufficient to provide enough light for the video recordings.

As it has been shown by von Helversen and Wendler (2000) another bushcricket species can use optical cues whenever available for orientation. Without landmarks the animals oriented poorly towards the speaker, in contrast to almost straight orientation with an additional light source. However, it is highly unlikely that our experimental conditions provided enough visual information to the orienting female for the following reasons: if we take crickets as a reference for the spectral sensitivity of orthopterans, they have three visual pigments, with peaks at 332, 445, 515 nm (Zufall et al. 1989); no orthopteran still known has a red pigment. Thus, if we take a realistic scenario—a green pigment with a peak at 520 nm—then there is almost no sensitivity at 640 nm (Zufall et al. 1989). Because our red light was dim, and with a spectrum above 650 nm, there was not enough light available for the orienting female. Furthermore, the speaker was hidden deep in the gap between the cones of the wall of the sound proof room. Thus, any visually guided orientation behaviour was highly unlikely.

In ten females which had been successfully tested in the free sound field the earphone system was applied. For this purpose females were anaesthetised with CO_2, the backpack was fixed to the pronotum and the ends of the flexible plastic tubes sealed into the spiracular openings. Special care was taken to prevent acoustic leakage due to incomplete sealing. Subsequent to each successful experiment the connection between tube and spiracle was controlled. One day following the earphone application, females were connected to the stimulation unit via three long, thin insulated wires (length 3 m, thickness 100 μm), providing the modified male calling song signal simultaneously to both ears at controlled IIDs. The wires were held by a stand to prevent any pulling force acting on the behaving animal. This set-up was placed in a small arena, where the turning movement of the female was monitored. To prevent females from any visual cues, their eyes were blinded by painting. Of the 10 females connected with the dichotic stimulation backpack 4 females could be tested at all 9 IIDs, and since at least 10 stimuli were applied for each IID, they responded without fatigue to about 100 stimuli. This required females to respond behaviourally for more than 15 min. With one further female we completed only four IID values, and received less than ten responses for the remaining IIDs, but the female then stopped responding. For the remaining females we still obtained some turning responses at various IIDs, but because they stopped responding before completion of all, these values are also not included in the figure, although they did not deviate from those presented in Fig. 4.

The four successful females performed phonotactic orientation by walking forward to the imaginary sound source, and turning to the side leading in interaural intensity by 1 dB and more. In contrast to the experiments in the free sound field, this time we measured the relationship between applied interaural IIDs and the subsequent turning of the insect to either side.

Since a turning of the female in the free sound field results in an increase of the effective SPL at one side, and unequivocally in a decrease at the opposite side, we mimicked this counter-current effect using an electronic device which was designed in such a way that pressing a switch reduced the SPL on one side by, e.g. 0.5 dB and simultaneously increased it on the other side by 0.5 dB, thus changing IIDs in steps of at least 1 dB. The sequence of applied IIDs was randomised.

Calibration of the earphones

For a dichotic ear stimulation the symmetry of the two speakers over the frequency range of interest is essential. Each pair of earphones was selected for symmetry by measuring the sound intensities at frequencies of 7 and 11 kHz (matching the major peaks in the stridulatory signal; see Fig. 1b), with a probe microphone (Bruel and Kjaer, Type 4182). The symmetry achieved was within 0.5 dB for these frequencies, and this symmetry was later encountered again in the lateralization behaviour of the animals (see Results, Fig. 4).

We had to confirm, that the same intensities were applied in the dichotic and in the free field experiments, by calibrating the earphones using a neurophysiological

method. First, the threshold of a unilateral omega-cell preparation (Römer and Lewald 1992; contralateral leg nerve carrying auditory afferents of the opposite ear cut) was determined for pure tone frequencies from 5 to 50 kHz in the free sound field ($n = 5$). The sensitivity was highest for frequencies between 15 and 20 kHz with a mean threshold of 25 dB SPL, increasing to 50 dB SPL at 5 kHz, and to 45 dB SPL at 50 kHz, thus confirming the broad frequency tuning of the homologous cell in all bushcrickets so far examined (e.g. Römer et al. 1989; Stumpner 2002). Next, we determined the threshold in response to the calling song in the same preparations (spectrum as shown in Fig. 1b), which was 41 dB SPL (± 2.3 dB). This higher threshold is due to the fact that the main energy in the song is well below 10 kHz, and that there is a steep increase of the omega-neuron threshold at these frequencies. Finally, the stridulatory signal (limited to 15 kHz) was also used in another set of preparations ($n = 5$) with the earphone applied to the ipsilateral side of the cell. This threshold value varied between different preparations by no more than 4 dB and was taken as reference for the dichotic behavioural experiments. We were thus confident that in both the free field and the dichotic experiments the intensity of the conspecific signal was similar, adjusted to a value of about 20–25 dB above the threshold of the omega-neuron.

Cross-talk between the ears

The unilateral omega-cell preparation was also used to test physiologically the cross-talk between the two ears in the dichotic stimulation paradigm. With both speakers applied to the tracheal tubes of the acoustic spiracles, the threshold of the unilaterally activated omega-cell was first tested for ipsilateral stimulation with pure tones between 5 and 15 kHz, and also for the calling song stimulus. Subsequently, the same stimulation was repeated via the earphone connected to the contralateral spiracle. Since the contralateral ear was functionally eliminated by cutting the afferent auditory fibres, the difference between both threshold measurements gives the cross-talk between the ears (Fig. 1c). The values varied between 47 and 74 dB for all frequencies and preparations ($n = 6$). More importantly, the cross-talk barrier for the calling song was of the same magnitude compared to pure tone stimulation (Fig. 1c). Thus, in the behavioural experiments with earphones the cross-talk barrier was 47 dB in the worst case, which is sufficiently large to guarantee that with the intensity used in the dichotic behavioural experiment of 65 dB SPL, each ear received sound from the opposite ear below 18 dB SPL, well below the hearing threshold. Thus we were confident to stimulate each ear independently from each other.

Directionality of the ear

It is generally accepted that IIDs determine the directional responses in the phonotactic approach of

orthopteran insects (for review, see Lewis 1983; Pollack 1998; Michelsen 1998; Robert and Göpfert 2002). In order to compare these binaural differences in the free sound field with the results of the dichotic experiments, the directionality of the ear was established neurophysiologically with the same calling song signal as being used in behavioural experiments. An unilateral omega-cell preparation was placed in the centre of an anechoic recording chamber with a speaker being rotated in steps of 10° at a distance of 50 cm in the horizontal plane around the preparation (for further details of the recording chamber, see Rheinlaender and Römer 1980). At each speaker position the threshold of the neuron (at least one action potential above spontaneous activity in three out of five stimulus responses) was established with an accuracy of ± 1 dB. By assuming that both ears have symmetrical directional characteristics, IIDs could be calculated for any direction of sound incidence.

Results

The phonotactic responses of ten females were first tested in the free sound field on the walking belt. Each
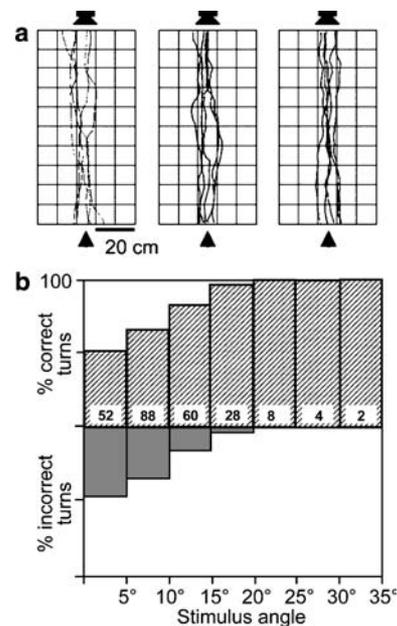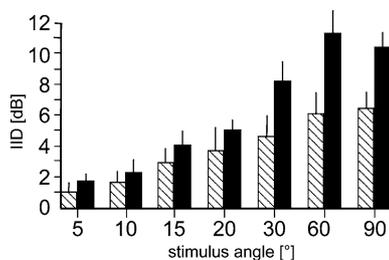

Fig. 2 a Phonotactic path of three females on the walking belt in response to the male calling song at 65 dB SPL, each over a virtual distance of 5 m. Superimposed walking tracks of a length of 1 m each. The position of the speaker is indicated on top, and the position of the starting point below (*arrowhead*). Note that females deviate only little from the midline, and that large stimulus angles are rare. **b** Percent of correct and incorrect turns at different stimulus angles, with a conspecific calling song at 65 dB SPL as stimulus. *Numbers* indicate number of observations. Accumulated data of five females, each analysed over a virtual distance of 3 m (number of stimulus-related turning responses, $n = 242$). Note that with a stimulus angle of $> 10°$ the turns become significantly correct, and with an angle of $> 25°$ no incorrect turns are made
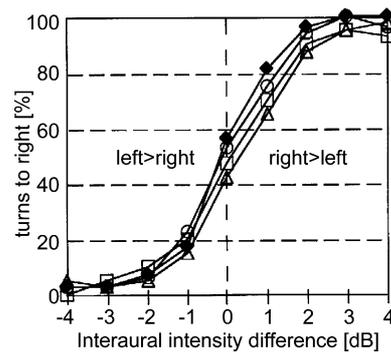
phonotactic approach was analysed over a virtual distance of 10 m. The females tracked the position of the speaker with a remarkable precision, deviating by no more than 10 cm in either direction from the ideal course (Fig. 2a). Females also tracked the sound stimulus faster with higher sound pressure levels; on average, females needed 26.4 s (± 4.7 SD; $n = 21$) for a virtual distance of 1 m at 80 dB SPL, but significantly longer (32.3 s ± 6.2 SD; $n = 18$) at 65 dB SPL. As a consequence of this straight path no case stimulus angles $> 35°$ occurred. In addition, the accumulated data demonstrate the probability of correct turns for different stimulus angles (Fig. 2b). Starting with stimulus angles of 6–10°, females turned significantly to the correct side (z test with Yates correction; $P < 0.01$), and with stimulus angles greater than 25° no incorrect turns were made. Only between 0 and 5° the probability of correct and incorrect turns was almost the same. From these results we can conclude that a stimulus angle $> 5°$ provides the insect with a reliable directional information in order to correct its phonotactic path.

Using the neurophysiologically determined directional characteristic of the hearing organ, we calculated the effective IIDs for any stimulus angle from these data, under the assumption of a bilateral symmetry of the auditory system (Fig. 3). For example, a sound incidence of 5° results in IIDs of only 1 dB with the full spectrum of the calling song. For stimulus angles of 10° this difference increases to 1.8 dB. It was shown in Fig. 2b that for stimulus angles of 6–10° the probability of correct turns becomes significant. Thus, a value of 1–2 dB IID is a significant value for the minimum interaural intensity resolution in this bushcricket. IIDs are larger, when only the high-frequency part of the calling song spectrum was used as stimulus (Fig. 3, black bars), with an average IID of 2.3 dB at 10°, and more than 10 dB with stimulus angles $> 60°$.

For highly motivated females in the dichotic experiments each sound stimulus elicited phonotactic behaviour towards the imaginary sound source, and a reliable turning movement to the louder side, when the IID was on an average 1 dB or more. Thus, the direction of the female's phonotactic path could be guided in a remote

Fig. 4 Results of behavioural dichotic stimulation experiments with four females, in which the interaural intensity difference of a 65 dB stimulus was varied in increments of 1 dB from 0 to 4 dB, with an advantage to either the left or right auditory side. The side leading in intensity as well as the amount of the difference was varied randomly. Note that with an IID of 1 dB there is a significant turn to the more strongly stimulated side, and with 2–3 dB difference very few incorrect turns are made. Each data point represents the percentage of turns to one side, taken from at least ten stimuli with the same IID

control manner by the operator. In fact, the phonotactic behaviour of the females in the dichotic stimulation paradigm resembled more of a remote control steering of a robot drive rather than a life insect, since a small difference in binaural signal intensity (see below) reliably elicited a turning to the side more strongly stimulated.

More than 100 dichotic calling song chirps could be applied to one individual without fatigue of the behavioural response. Thus, in the dichotic stimulation experiments the resolution of IIDs could be precisely demonstrated for four animals (Fig. 4). The curves demonstrating the dependency of the turning behaviour on IIDs are remarkably similar for all females with respect to the steepness and saturation level. The results of another female, which are not included in the figure because we obtained less than ten stimulus-related turning responses for the nine IIDs tested are nevertheless also consistent with the data of the other four females, in that a difference of 1 dB on an average elicited a turn to the louder side. With IIDs of 2 dB females turned in about 90% of the stimuli to the side leading in intensity, and with IIDs above 3 dB the saturation is reached. In three out of four animals the 75% level (25% level for turns to the left side) of correct performance was already given with an IID of 1 dB. The crossing points of the curves (50% turns to either side) are equally distributed around an IID of 0 dB, indicating individuals with only slight auditory handedness. However, the data indicate neither a bias in the sample of animals nor in the configuration of the dichotic earphone system.

Fig. 3 Binaural intensity differences in the auditory system of *G. gratiosa* at various stimulus angles, derived from the directionality of five monaural preparations, under the assumption of symmetry between both sides. *Hatched bars* the stimulus was a calling song with the full frequency spectrum; *black bars* calling song with only the high frequency part of the spectrum high-pass filtered; cut-off frequency 11 kHz. For further explanation see text

Discussion

In both vertebrates and insects, sound localization is usually based on a binaural comparison, as it can be

most easily demonstrated in the orientation responses of unilaterally deafened moths, grasshoppers and crickets (Roeder 1967; Ronacher et al. 1986; Moiseff et al. 1978; Pollack et al. 1984). The results of these experiments indicate a rather simple strategy for positive (or negative) phonotactic responses of turning towards (or away from) the more strongly stimulated ear, given that the temporal pattern of the stimulus is preserved in the discharge of ascending interneurons (Stabel et al. 1989). If, as an exception, one-eared female crickets showed successful phonotactic orientation behaviour, this could be attributed to plasticity in the structure of primary afferents and function of central neurons after the loss of one hearing organ (Schmitz et al. 1986; Huber 1987, 1990; Schildberger 1994). In a few cases of successfully orienting crickets, where no neuronal regeneration could have taken place, it was hypothesized that their orientation requires a sequential comparison of the acoustic input before and after a turn. Such a mechanism has been demonstrated experimentally in an ultrasound sensitive moth (Greenfield et al. 2002).

Since in most vertebrates and humans interaural intensity and time differences can be used for calculating the direction of a sound source, the distance between the ears of insects is so small that differences in the time-of-arrival of the sound wave are in the range of 10–30 μs, which makes it highly unlikely that the insect's central nervous system can make use of such small time differences. Thus, behavioural directional responses must rely on IIDs enhanced by the peripheral directional characteristics of the hearing system. A rare exception is the parasitoid fly *Ormia ochracea* (Diptera: Tachinidae) with a microscale ear. The fly is able to accurately localize sound sources within 2° azimuth. Mason et al. (2001) provide evidence that ITDs in the order of 50 ns can be used, owing to a combination of the mechanical system which increases the spike latencies more than 100-fold compared to the IIDs and a hyperacute timing of a large ensemble of afferent spikes, to discriminate such small differences in source localization.

In bushcrickets, the directionality of the ear is the result of a sophisticated anatomical arrangement of the hearing organ in the tibia of the foreleg, in conjunction with a tracheal tube which connects the inner surface of the ear drum with the lateral surface of the body wall through a spiracular opening. This trachea acts as a sound guide, and its specific geometry increases the sound pressure at the inner surface of the ear drum by more than ten times compared to the external surface, depending on sound frequency (reviews in Lewis 1983; Bailey 1991; Michelsen 1998). Thus, whereas diffraction of sound by the legs does not provide the directional cues for sound localisation (at least not at the frequencies of the callings songs), as a result of the anatomical arrangement of the spiracular openings the directionality of the ear is based on diffraction and attenuation of the sound waves at the position of the spiracular opening at the lateral body surface (Michelsen et al. 1994; Michelsen 1998).

The peripheral directionality thus achieved is large enough to guide females reliably in their phonotactic approach to a sound source broadcasting conspecific calling songs. The phonotactic movements on the walking belt exhibit a rather direct course with only little deviations from the ideal path (Fig. 2a), so that stimulus angles greater than 35° never occurred. But even stimulus angles as small as 6–10° provided the females with sufficient directional information based on IIDs, and they turned significantly to the more strongly stimulated side. No incorrect turns were made with stimulus angles larger than 25°. However, the IIDs at such small stimulus angles are also small, when studied with the natural calling song spectrum (1 and 1.8 dB at 5 and 10°, respectively). This is due to frequency-dependent sound diffraction at the location of the spiracular opening. Larger IIDs can be established only with pure tones of the high frequencies within the song (20 dB total right–left difference; Shen 1993) or with high-pass (10–40 kHz) filtered (1.8 and 2.1 dB at 5 and 10°, and more than 10 dB at stimulus angles of 60° and more, respectively).

Our new dichotic ear stimulation technique enabled us to clearly establish in a behavioural experiment the smallest IID, that can be used by this female bushcricket for a reliable phonotactic approach. Based on a cross-talk barrier of at least 47 dB with the earphone system, we could apply lateralized stimuli to either hearing organ in a freely moving animal without stimulating the opposite one in a wide range of intensities. Thus, even with sound pressure levels of 65 dB SPL, where the behavioural experiments with the earphones have been performed, the resulting SPLs at each ear due to contralateral stimulation remained at less than 20 dB SPL, and thus below the absolute hearing thresholds of females for pure tones (between 20 and 25 dB SPL; Shen 1993; Kalmring and Jatho 1994; and own measurements) and well below the thresholds in response to the natural calling song.

The results obtained with the dichotic ear stimulation reveal a remarkable sensitivity for small IIDs in the order of 1–2 dB. At IIDs of 1 dB all females turned on an average to the side more strongly stimulated, and with IIDs of 2 dB they turned in about 90% of the stimuli to the side leading in intensity. Similar values have been found in humans (0.5 dB, Mills 1958), mammals and barn owl (about 1 dB; Wakeford and Robinson 1974; Konishi 1983) and monkeys (2–3 dB; Wegener 1974; Houben and Gourevitch 1979). The binaural intensity resolution of *G. gratiosa* is also in good agreement with that of a shorthorned grasshopper (*Chorthippus biguttulus*; von Helversen and Rheinlaender 1988). How grasshoppers and bushcrickets achieve a resolution of such small IIDs of 1–2 dB remains currently unknown and deserves further neurophysiological analysis.

The ability to resolve such small IIDs is in good agreement with the quantitative data on the accuracy of the phonotactic approaches, where females do not deviate with their longitudinal body axis to the acoustic

target by more than 35° on the walking belt. Thus, they experience only small stimulus angles, which in turn create only small IIDs in the order of 1–2 dB at stimulus angles of 6–10°, and less than 5 dB at 30°. The data are also consistent with those on the accuracy on sound localization in crickets, where the minimum audible angle in azimuth in front of the animal has been determined with 15° (Rheinlaender and Blätgen 1982) and about 11° (Wyttenbach and Hoy 1997). The binaural IIDs for such angles are about 2 dB (Rheinlaender 1984). A recent behavioural analysis of cricket steering with a fast trackball system has also revealed the minimum binaural cues: when phonotactically walking female crickets were exposed to binaural song patterns of different SPL, they steered significantly to the louder source when the intensity difference was greater than 1 dB (Hedwig and Poulet 2005). Such relatively weak binaural directional cues in *G. gratiosa* result from the unusual low-carrier frequencies in the calling song, with maximum energy between 3.5 and 7 kHz. For these low frequencies (and corresponding large wavelengths), the diffraction-based directionality at the position of the spiracular opening at the lateral body wall is rather weak, and the IIDs are smaller than those obtained with the high frequency portion of the spectrum alone (compare Fig. 3), or with pure tones of 15 or 25 kHz (Shen 1993). For females performing phonotaxis under natural conditions in a complex habitat and at some distance from a male only low frequencies are available due to frequency-dependent attenuation (Michelsen 1978; Michelsen and Larsen 1983; Römer and Lewald 1992; Schul and Patterson 2002). Furthermore, directional cues in natural habitats may be strongly degraded, depending on the position of signaller and receiver, intervening vegetation and a variety of other properties (Rheinlaender and Römer 1986; Römer and Lewald 1992; Michelsen and Rohrseitz 1997; Gilbert and Elsner 2000). It is therefore reasonable to assume that the high performance of bushcricket and grasshopper auditory systems to make use of small IIDs is an important adaptation for their success in phonotactic mate finding in nature.

A still open question for directional hearing in insects is, how such behaviourally relevant IIDs of 1–2 dB are reliably encoded into discharge differences of afferents and interneurons of the auditory pathway. A common mechanism for processing small IIDs at the central nervous level is contralateral inhibition, which enhances subtle peripheral differences into large discharge differences of bilaterally homologous neurons (review by Pollack 1998). There is evidence from dichotic ear stimulation techniques in a neurophysiological approach, that IIDs of 2 dB result in reliable coding of sound direction in a pair of directional sensitive interneurons (Rheinlaender 1984). Ongoing research with a dichotic stimulation in bushcrickets is dedicated to this problem.

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