The heterospecific calling song can improve conspecific signal detection in a bushcricket species

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Abstract

In forest clearings of the Malaysian rainforest, chirping and trilling *Mecopoda* species often live in sympatry. We investigated whether a phenomenon known as stochastic resonance (SR) improved the ability of individuals to detect a low-frequent signal component typical of chirps when members of the heterospecific trilling species were simultaneously active. This phenomenon may explain the fact that the chirping species upholds entrainment to the conspecific song in the presence of the trill. Therefore, we evaluated the response probability of an ascending auditory neuron (TN-1) in individuals of the chirping *Mecopoda* species to triple-pulsed 2, 8 and 20 kHz signals that were broadcast 1 dB below the hearing threshold while increasing the intensity of either white noise or a typical triller song.

Our results demonstrate the existence of SR over a rather broad range of signal-to-noise ratios (SNRs) of input signals when periodic 2 kHz and 20 kHz signals were presented at the same time as white noise. Using the chirp-specific 2 kHz signal as a stimulus, the maximum TN-1 response probability frequently exceeded the 50% threshold if the trill was broadcast simultaneously. Playback of an 8 kHz signal, a common frequency band component of the trill, yielded a similar result. Nevertheless, using the trill as a masker, the signal-related TN-1 spiking probability was rather variable. The variability on an individual level resulted from correlations between the phase relationship of the signal and syllables of the trill. For the first time, these results demonstrate the existence of SR in acoustically-communicating insects and suggest that the calling song of heterospecifics may facilitate the detection of a subthreshold signal component in certain situations. The results of the simulation of sound propagation in a computer model suggest a wide range of sender-receiver distances in which the triller can help to improve the detection of subthreshold signals in the chirping species.

Keywords

Stochastic resonance; Signal detection; Auditory interneuron; Acoustic communication; Ensiferan insects

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1 Introduction

In the nocturnal tropical rainforest, many insect and anuran species communicate via airborne sound simultaneously. In this situation, which is somewhat like that of a cocktail party, the background noise level is usually high (Lang et al., 2005; Riede, 1997; Ellinger and Hödl, 2003; Hartbauer et al., 2010; Römer, 2013), and receivers must face challenges while listening and responding to the signals of conspecifics due to potential masking interference (Bee and Micheyl, 2008). Signalers usually evaluate the temporal signal pattern to discriminate conspecific from heterospecific signals, but this task is difficult to perform when a multitude of heterospecific signals degrade the amplitude modulations of calling songs that contain information about species identity. Some animals have found a solution to this problem in that the frequency of their calling songs matches the sensitivity of the receiver (Schmidt and Römer, 2011; Schmidt et al., 2012). However, this type of frequency tuning in receivers is less effective in communication systems whereby the signaler generates songs with more broadband frequencies. This is exactly the case in many bushcricket species in which the males attract phonotactically-responding females from a distance.

In the genus *Mecopoda*, several sibling species are morphologically similar, but these can easily be distinguished by differences in their species-specific calling song patterns (Nityananda and Balakrishnan, 2006; Siegert et al., 2011; Schneider and Römer, 2016). Two *Mecopoda* species live sympatrically in forest clearings of the Malayan tropical rainforest: a trilling species and a chirping species, the males of which advertise themselves by producing periodic signals with frequency compositions that strongly overlap those of the songs of the trilling species (Fig. 1). Since both species are active at the same time (i.e., after sunset), and the calling songs of the trilling species are of high intensity, it was expected that the trilling species' song would mask the calling song of the chirping species. On the contrary, Siegert et al. (2013) demonstrated that a rather low frequency component at 2 kHz allowed males to establish synchronous entrainment even when the trill of the sympatric *Mecopoda* species was broadcast 8 dB louder than the conspecific calling song. This frequency component is weak in the calling song of the trilling species but rather high in the chirping one. Surprisingly, Siegert et al. (2013) found an improvement in the detection of periodic 2 kHz signals in the response of an auditory neuron (TN-1) when the heterospecific masking trill was broadcast simultaneously. This unexpected result may be attributed to a phenomenon known as stochastic resonance (SR), which explains noise-enhanced signal detection due to the resonance of random and uncorrelated noise with the signal (Benzi et al., 1981; Benedix et al., 1994; Wiesenfeld and Moss, 1995).

SR refers to a paradox phenomenon that improves the sensitivity of a system to external stimuli at rather weak levels of noise (e.g., Collins et al., 1995, 1996; Gluckman et al., 1996; Gammaitoni et al., 1998; Russell et al., 1999; Henry, 1999; Tougaard, 2000; Ward et al., 2002; Lyttle, 2008). Noise is usually considered to be something detrimental that should be minimized whenever possible; however, noise is enhancing the detection of weak periodic signals in certain cases. Therefore, McDonnell and Abbott (2009) described SR as a "noise benefit in a signal-processing system", or "noise-enhanced signal processing". Low levels of stochastic noise usually improve the detection of subthreshold signals, while higher noise

levels adversely affect signal detection because signals are masked by noise (e.g., Collins et al., 1996; Gammaitoni et al., 1998; Henry, 1999).

To date, SR has been found in many different receiver systems, either of biological or technical origin. SR has generally been found in a nonlinear input-output system (for an exception, see Fuli ski and Góra, 2000) when signals are broadcast at subthreshold levels (but see Collins et al., 1995) and moderate levels of noise are added. The detection of subthreshold, periodic signals is improved, which reduces the probability of missing signals by increasing the hit rate at the same time according to signal detection theory (Tougaard, 2002). SR can be investigated by studying the receiver's ability to detect signals during steadily-increasing noise levels.

Several studies have shown that SR can improve signal detection in various organisms. For example, SR seems to improve the sensitivity of mechanoreceptor hair cells in the crayfish (Douglass et al., 1993). SR was also found in a study by Russell et al. (1999), who investigated the feeding behavior of paddlefish. Their results demonstrated that the success of capture rate was increased in the presence of low electrical background noise levels. Moreover, Levin and Miller (1996) conducted SR experiments to examine the cercal system of crickets. They believe that adding noise to a weak periodic air flow is improving the detection of predatory wasps. SR was additionally found to play a role in the mating system of the stink bug *Nezara viridula*, in which it improved the detection of weak vibratory signals in a noisy environment (Spezia et al., 2008). As another positive effect, SR was found to improve the nervous processing of auditory information in the brains of frogs. In this case, the response of midbrain auditory neurons to a weak periodic input signal was enhanced in the presence of broadband noise (Ratnam and Feng, 1998; Bibikov, 2002).

Currently, it is unclear whether SR plays a vital role in acoustically-communicating organisms that live in habitats that have rather high levels of background noise such as the nocturnal tropical rainforest. One reason for this lack of knowledge is that many SR studies use white noise as a masker because the characteristics of this artificial noise strongly deviates from natural signals that are characterized by a species-specific spectral content as well as temporal structure. Furthermore, the noise intensity must be moderate to favor SR, which is not necessarily the case when heterospecific signalers are in close proximity to one another or when their signals are of high intensity. In order to study whether SR improves the acoustic communication in *Mecopoda*, we studied the response to subthreshold, species-specific 2 kHz signals in the nervous system of individuals of the chirping *Mecopoda* species while increasing the amplitude of the song of the trilling heterospecific *Mecopoda* species. To compare our results with those of previous SR studies, we also broadcast increasing levels of white noise together with subthreshold signals. We recorded signals from an afferent auditory neuron with T-shaped morphology (TN-1; Suga and Katsuki, 1961; McKay, 1969) because it has been shown to reliably encode conspecific chirps even under conditions of natural background noise (Siegert et al., 2011). This allowed us to study SR with only little influence of internal neuronal noise, which can be crucial for the effectiveness of SR (Aihara et al., 2008, 2010; Krauss et al., 2016).

2 Materials and Methods

2.1 Insects

We included males and females of a chirping song variant of the genus *Mecopoda elongata* (Orthoptera, Ensifera, Tettigoniidae, Mecopodinae) in our study, which had originally been described as species S by Sismondo (1990). Insects were taken from a laboratory breed, founders of which had been originally collected in Malaysia in the years 2010 and 2011 (Ulu Gombak, Selangor, Kuala Lumpur). Males of this species produce calling songs that consist of chirps repeated at a period of 2 s (27°C ambient temperature). In a chorus, males of this species tend to produces chirps in synchrony. In the breeding room, insects were exposed to a light:dark cycle of 12:12 h and maintained at a constant temperature of 27°C, 70% relative humidity. Their diet consisted of fresh lettuce, apples, fish food and oatmeal. There is no publication so far that describes the trilling *Mecopoda* species morphologically, but Korsunovskaya (2008) described this species acoustically as "Mecopoda sp. 4". One male and female voucher specimen of the triller were deposited at the National History Museum in Vienna (NOaS-11/2013).

2.2 Neurophysiology

SR was studied at the level of a first-order auditory interneuron ascending to the brain. Because this neuron has a T-shaped morphology, it is described as TN-1 neuron (Suga and Katsuki, 1961). Before dissection, insects were anesthetized with Chloroethylene gas. The legs (except the forelegs), antennae and wings were removed, and individuals were mounted ventral side up to a platform using dental wax. The metathoracic ganglion was also removed to avoid the neuronal activity that is normally generated by the flight oscillator and input from the cercal organ. The connectives between the metathorax and the first abdominal segment were cut, and a small piece of paper was inserted into the abdomen to relieve hemolymph pressure. The leg opposite the auditory stimulus was removed to avoid any contralateral inhibition of the TN-1 response. Subsequently, the cervical connectives were exposed, and the right connective was lifted using a tungsten-wire hook electrode. After removing the hemolymph, both connectives were cut between the suboesophageal ganglion and prothoracic ganglion. The neck was covered with petroleum jelly (Vaseline) to prevent the desiccation of the connectives. Signals recorded with the electrode were amplified against an indifferent silver electrode that had been inserted into the abdomen (Suga and Katsuki, 1961; McKay, 1969). The experiments were performed in an anechoic chamber in which two loudspeakers were positioned adjacent to one another, 24 cm away from the insect preparation. The insect holder was placed on top of a heating platform (G. Maier, Electrotechnik GmbH) which was maintained a constant temperature of about 27 °C at the position of the insect.

The neuronal response was amplified via a custom-made amplifier that was fabricated as described by Land et al. (2001). Analog to digital conversion was made using a Powerlab/4s (AD Instruments), and converted data were saved in Chart (Version 5.5.6, AD Instruments, Spechbach, Germany).

2.3 Acoustic stimulation

Acoustic stimuli consisted of triple-pulsed pure tones (further referred to as signal) and were presented in several signal and noise combinations: 1) a triple-pulsed 20 kHz signal was presented together with white noise to test for the existence of SR at the carrier frequency at which the TN-1 neurons were tuned (Siegert et al., 2013), 2) a triple-pulsed 2 kHz signal and white noise, 3) a triple-pulsed 2 kHz signal and a trill of a heterospecific *Mecopoda* species that lacked this frequency band and 4) a triple-pulsed 8 kHz signal and a *Mecopoda* trill with high energy at this frequency band (see Fig. 2). The signal period was limited to 2 s in order to mimic the natural chirp period observed at an ambient temperature of 27 °C (Sismondo, 1990; Siegert et al., 2013). Noise was broadcast in loop mode, and care was taken that the phase relationship between the signal and noise was changed in a random manner. With the exception of the trill, all signals were generated in Cool Edit Pro 2.0 (Syntrillium Software Corporation, Phoenix, AZ, USA).

2.4 Playback

For acoustic playback, Cool Edit Pro was used to control an external audio interface for D/A conversion (RME Fireface UC, Haimhausen Bavaria, Germany). Analogue output signals were attenuated via a two-channel programmable attenuator (PA5, Tucker Davis Technologies, Alachua, FL, USA) and amplified using a NAD stereo power amplifier (C275BEE, NAD Electronics International, Canada) with a flat frequency response up to 100 kHz. Acoustic signals were broadcast via two leaf tweeters that exhibited a rather flat frequency response between 1 kHz and 45 kHz (EAS-10TH400A, Technics, Kadoma, Osaka, Japan).

2.5 Sound calibration

The SPL of playback signals were calibrated at the position of the insect preparation with a calibrated microphone (model 2450, Larson Davis Laboratories, USA) that was connected to a sound level meter (CEL 414, CEL Instruments Ltd. Hitchin, Herts, England, attached to a filter unit CEL 296). Sound calibration of pulsed signals was carried out in the fast reading mode by broadcasting sound signals in the loop mode. The sound level meter operated with a flat frequency response in a range between 100 Hz and 45 kHz. Although we also recorded the peak values of pulsed signals, the signal to noise ratios given in the results refer to averaged values obtained in the fast reading mode. Sound signal amplitudes were calibrated relative to 20 μ Pa.

2.6 Stochastic resonance experiments

The thresholds of the pure tone signals were determined by systematically changing the signal intensity until 50% of the signal presentations elicited a TN-1 response of at least one spike per signal presentation. The SPL of the signal was then decreased by 1 dB (1 dB subthreshold). If the SR increased the detection of the subthreshold signal, noise added to the signal would increase the TN-1 response probability to a value higher than 50%. To quantify the effect of noise on the spiking response of TN-1 to pure tone signals, the noise was steadily increased in steps of 2 or 3 dB after the presentation of a sequence of 35 identical pure tone signals (2 s signal period). SNR values refer to the dB difference between

the signal and noise amplitude. To exclude a possible effect caused by neuronal adaptation processes, the responses to five signal presentations at the beginning of a playback sequence were excluded from data evaluation. Results represent the proportion of signal presentations that elicited at least one spike during a sequence of 30 stimulus presentations. Since the phase relationship between the pulses of the signal and the trill differed between stimulus sequences, we assumed that the signal detection rate not only depended on the SNR, but also on the phase lag between the signal and the syllables comprising the trill. Therefore, we also quantified the variability of the TN-1 response on an individual level at several SNRs by repeating the sequence of the pure tone signals 10 times at a given SNR. In this experiment, the intensity of the noise was increased in steps of 10 dB.

2.7 Data evaluation

TN-1 recordings were exported to Spike2 (V5.21, Cambridge Electronic Design) to run a custom-written evaluation script which counted the instances of presence or absence of TN-1 related spikes in a time window of 100 ms during the signal presentation, taking a response latency of 10 ms into account. The extracellular potentials of TN-1 neurons are of high amplitude so TN-1 activity can be easily discriminated from other neuronal activity by setting a user-defined amplitude threshold. With the exception of signal onset, white noise rarely elicited a spiking response, whereas the presentation of the trill at low SNRs frequently elicited spikes. In order to quantify the trill-related TN-1 responses, we also counted the number of spikes that occurred in a time window of 1 s before the signal onset. To convert this noise-related spike count for a given SNR into a spiking probability, the average number of noise-related spikes was divided by 10 to obtain the same time basis as the stimulus (100 ms), and the result was multiplied by 100. Individuals with a trill-related spiking probability that exceeded 30% were excluded from the SR analysis. Since spike intervals shorter than 100 ms may lead to confusion between noise-related spikes and signal-related spikes, we also evaluated the probability of trill-related, interspike intervals (ISIs). Generally, noise-related ISIs shorter than 100 ms rarely occurred and, therefore, we used the first method to quantify the trill-related spiking response. In additional experiments, we studied the influence of the phase relationship between the pure tone pulses and the first soft syllable of the trill on TN-1 response (Fig. 3). The correlation between the phase lag and TN-1 response obtained from single individuals was tested for significance in Sigma Plot (v. 13.0, Systat Software, Inc.).

2.8 Modelling sound propagation

The sound propagation of pure-tone signals was simulated in NetLogo (v. 5.3.1) to estimate the spacing between conspecific and heterospecific signalers in which SR was likely to occur. Simulations of the active space were based on realistic sound propagation properties observed in the field (Römer and Lewald, 1992) and the hearing threshold of the receivers was taken into account. Signal attenuation over a distance was simulated using equation (1) (see below) for a pure tone of carrier frequency of 5 kHz to simulate the relatively low frequency bands that are contained in the chirp. The signal attenuation of the trill was simulated by modelling a pure tone with a carrier frequency of 10 kHz using equation (2) (see below) because this frequency is similar to a prominent frequency band in the trill. The SPL of the receiver was set to 81 dB at a distance of 1 m, a value that corresponds to the

average peak SPL of *M. elongata* males measured at a distance of 1 m (Hartbauer et al., 2014). The amplitude of the 10 kHz signal was simulated assuming an amplitude of 85 dB SPL and measured at a distance of 1.2 m (corresponds to 103 dB measured at 15 cm; see Krobath, 2013). A hearing threshold of 52 dB SPL (–1 dB subthreshold) was simulated for the 5 kHz signal which corresponds to the hearing threshold for 2 kHz signals. A hearing threshold of 40 dB SPL was defined for the 10 kHz signal which corresponds to the TN-1 threshold of the 8 kHz signal.

$$I = 81\,dB - [10.05 * ln(x) - 0.865] \quad (1)$$

$$I = 85\,dB - [14.18 * ln(x) - 0.891] \quad (2)$$

2.9 Statistics

If SR improves signal detection, we should expect a higher number of relative TN-1 responses exceeding the 50% threshold at moderate SNRs compared to very high SNRs (noise amplitude is low compared to the amplitude of the subthreshold signal). This was tested by performing z-tests with Yates correction, which tests the proportions for statistically significant differences taking the sample number into account. Data used to perform the z-test are indicated by dashed boxes in Figs. 4 and 6. All statistical tests were performed in Sigma Plot version 13 (Systat Software Inc.).

3 Results

3.1 White noise

The average threshold required to elicit a TN-1 response to 50% of the 20 kHz signals was 35 ± 4.4 dB SPL (mean $\pm$ SD; N = 10). The percentage of TN-1 responses to 30 repetitions of this signal, presented 1 dB subthreshold, frequently exceeded the 50% value when white noise was broadcast at SNRs between +16 and –7 dB (Fig. 4A). Exceeding the threshold occurred significantly more often at moderate SNRs compared to high SNRs (see dashed boxes in Fig. 4A; $p < 0.05$, N = 10, z-test). However, at SNRs lower than –6 dB, the percentage of TN1 responses decreased remarkably. Since the TN-1 neuron is tuned to frequencies higher than 10 kHz (Siegert et al., 2013), the average hearing threshold for triple-pulsed 2 kHz signals was much higher (52.6 ± 3.2 dB SPL). The percentage of TN-1 responses to 2 kHz signals broadcast at 1 dB subthreshold exceeded the 50% threshold significantly more often at moderate amplitudes of white noise (indicated by dashed boxes in Fig. 4B; $p < 0.05$, N = 10, z-test) and strongly declined at SNRs less than +5 dB (Fig. 4B). The between-individual variability of TN-1 responses was higher during presentations of the 2 kHz signal as compared to the 20 kHz signal. These results indicate that white noise improved signal detection more reliably. To compare the TN-1 responses of a single individual to repeated presentations of the same stimulus sequence, we broadcast either 2 kHz or 20 kHz signals 10 times at certain SNRs. The results obtained from two individuals are shown in Fig. 5, the detection rate of 20 kHz signals increased significantly at various

levels of white noise (p < 0.05, Mann-Whitney *U* test followed by a Tukey post hoc test), whereas the detection ability for 2 kHz signals improved only marginally at all tested SNRs in one individual and was significantly higher at a SNR of 22.8 dB in another individual.

3.2 Triller song

When the heterospecific trill was broadcast at the same time as the 2 kHz signal, the TN-1 responses were variable between different individuals. Therefore, the average percentage of TN-1 responses obtained from 10 individuals remained below 50% at various SNRs when the 2 kHz signal was broadcast at 1 dB subthreshold (Fig. 6A). At SNRs between +15 dB and 0 dB (right dashed box in Fig. 6A), the percentage of the TN-1 responses was high in some individuals and low in others (indicated by the large error bars and the dashed lines in Fig. 6A). Nevertheless, the proportion of TN-1 responses exceeding the 50% threshold was significantly higher in this range of SNRs compared to high SNRs at which the trill amplitude was very low (left dashed box in Fig. 6A; p < 0.05, N = 10, z-test). The trill exceeded the average hearing threshold of receivers at a SNR of 12 dB (vertical line above the graph in Fig. 6A). At SNRs lower than −5 dB, however, the average percentage of TN-1 responses decreased monotonically. The average hearing threshold for the 8 kHz triple-pulsed signal was 40 ± 5.7 dB SPL. Presenting this signal one dB subthreshold together with the trill resulted in a significant increase of relative TN-1 responses exceeding the 50% threshold at SNRs between 0.8 and −5 dB compared to high SNRs (Fig. 6B; p < 0.05, N = 10, z-test). This range of SNRs is close to the hearing threshold of the trill (−3 dB, vertical line above the graph in Fig. 6B). At SNRs less than −8 dB, the average proportion of the TN-1 responses decreased gradually.

3.2.1 Phase relationship between signals and the trill—We examined the TN-1 response variability on the level of a single individual because the TN-1 response to the 2 kHz triple-pulsed signal showed rather high levels of variability at SNRs at which the heterospecific trill exceeded the hearing threshold (see Fig. 6A). The TN-1 response to the 2 kHz and 8 kHz signal obtained from a single individual was variable as indicated by the error bars shown in Fig. 7A and C. A significant increase in TN-1 response was only found when 2 kHz signals were presented at a SNR of 14.8 dB (p < 0.05, Mann Whitney *U* test followed by a Tukey post hoc test). By plotting the TN-1 responses against the phase lags and referring to the time separating the onset of the signal and the next soft syllable of the trill, we could reveal a significant negative correlation between both parameters (2 kHz: p < 0.05, Spearman rank order correlation, cc = −0.510; 8 kHz: p < 0.05, Spearman rank order correlation, cc = −0.556; Fig. 7B and D). We observed the highest TN-1 response (the red dot in Fig. 7B) where a time lag of 11.8 ms separated the onset of signal and the next soft syllable of the trill (Fig. 7E). On the contrary, a time lag of 21.3 ms resulted in the lowest percentage of TN-1 response (blue dot in Fig. 7F). Time lags that were nearly the same resulted in the highest and lowest percentages of TN-1 response when the 8 kHz signal was broadcast together with the trill as shown in Fig. 7D. These results demonstrate that a temporal overlap between a higher number of loud syllables and the triple-pulsed signal favored a TN-1 response, whereas the overlap of soft syllables diminished it (Fig. 7E and F).

3.3 Occurrence of stochastic resonance

The presence or absence of SR is shown in Fig. S1. In a large proportion of individuals, SR improved the TN-1 response probability at moderate amplitudes of white noise (Fig. S1, blue bars). In some individuals, the percentage of TN-1 response remained below 50% at all tested SNRs, which demonstrates the absence of SR (Fig. S1, red bars). We also evaluated the proportion of individuals for which the trill-related spiking activity exceeding 30% (see materials and method section for the calculation of the trill-related spiking response, Fig. S1, green bars). In contrast to the trill, white noise rarely elicited spiking activity in the interval between subsequent signal presentations.

4 Discussion

In the current study, the presence of stochastic resonance (SR) could be clearly demonstrated in the auditory system of a chirping *Mecopoda* species, as evinced by the percentage of the TN-1 response that exceeded the critical detection threshold of 50% at moderate levels of white noise. However, broadcasting high noise amplitudes resulted in a strong decrease in signal detection due to masking interference with the signal (Hänggi, 2002). In its natural habitat, the chirping species is confronted with noise from a multitude of sources. The song of the heterospecific trilling *Mecopoda* species has especially important implications for intraspecific acoustic communication among chirping species due to similarities in the frequency content of its calling songs. Siegert et al. (2013) showed that the 2 kHz band in the conspecific signal is essential for acoustic communication when a heterospecific trilling species is active at the same time. As shown in Figs. 4 and 6, broadcasting white noise and the heterospecific trill improved the detection of subthreshold signals up to a critical SNR at which the high noise levels masked the signal. This optimum level of white noise and its effect on the detection of signals has been described in many studies and is known as the stochastic resonance peak (see review by McDonnell and Abbott, 2009).

Simultaneously broadcasting white noise clearly improved the detection of the 20 kHz signal, but also resulted in high levels of variability in TN-1 responses to the 2 kHz signal presented at moderate amplitudes of white noise (Fig. 4). Differences between the results obtained with white noise and the trill may be due to the tuning characteristics of the TN-1 neuron (Zhantiev and Korsunovskaja, 1983; Hartbauer et al., 2010) which favor the detection of the 20 kHz signal but not the 2 kHz signal. The average hearing threshold of individuals included in our study was 35 ± 4.4 dB SPL for the 20 kHz signal, 52.6 ± 3.2 dB SPL for the 2 kHz signal and 40 ± 5.7 dB SPL for the 8 kHz signal. Siegert et al. (2013) showed that TN-1 neurons are less sensitive to frequencies lower than 10 kHz, even though the 2 kHz band in the conspecific signal is essential to maintain acoustic communication when the heterospecific trilling species is simultaneously active. As a result of this frequency tuning, 2 kHz signals suffer from masking interference at much higher SNRs (<+5 dB), whereas masking of the 20 kHz signal was observed at SNRs less than −6 dB (Fig. 4). These TN-1 tuning characteristics may explain the stronger increase in signal detection rate observed when 8 kHz rather than 2 kHz signals were broadcast together with the trill at SNRs between 0 dB and −5 dB (Fig. 6B). Furthermore, the low sensitivity of TN-1 neurons to the 2 kHz signal may cause a temporal threshold shift in the TN-1 neuron when white

noise is broadcast at higher amplitudes. Due to this adaptation process, we rarely observed TN-1 responses to white noise except at noise onset. We observed relatively weak improvements in the ability of the individuals to detect 2 kHz signals when broadcast together with white noise (compare Fig. 4A with 4B and also see Fig. 5), and such a noise-dependent threshold shift may be responsible for this. This may be due to the fact that the level of noise was higher at SNRs in which we found an increase of signal detection in the 2 kHz playback experiment.

Simultaneously broadcasting pure-tone signals and the trill resulted in high levels of variability in TN-1 responses, which is clearly revealed by the high standard deviations shown in Fig. 6. We propose two explanations for this result: First, the trill consists of a stereotyped sequence of syllables with a frequency composition that is broadband, but far from random with respect to its frequency content, the cross-correlations of the frequencies and the temporal signal pattern. This difference between the trill and white noise may limit the possibilities for SR. Second, the TN-1 response strongly depended on the time lag between the pulse onset of the signal and the next soft syllable of the trill (Fig. 7B and D). The highest TN-1 response occurred when the pulses of pure tone signals overlapped with a higher number of loud syllables of the trill (Fig. 7E), indicating that any improvement in signal detection strongly depended on the precision of the pulse and syllable timing.

It is still unclear whether the moderate SR detected is a consequence of central nervous mechanisms or the non-linear properties of the tympanal membrane, because auditory function can be enhanced on the periphery in the presence of low or moderate noise levels under certain circumstances (Henry, 1999; Indresano et al., 2003; Nadrowski et al., 2004). The dependence on the exact timing of signal pulses and syllables of the trill suggests that a peripheral mechanism could account for the moderate increase in the signal detection rate obtained with the 2 kHz signal, particularly because the ears of insects (energy detectors) have short integration time constants between 4 and 25 ms (Tougaard, 1998) that increase with the bandwidth of the noise (see Tougaard, 2000). In contrast, central nervous processing has longer integration time constants (>200 ms; Ronacher et al., 2000).

4.1 Biological relevance of SR

In a theoretical receiver model, Tougaard (2000) came to the conclusion that the range of usable noise levels that lead to SR are limited, which leads researchers to question the biological relevance of SR (but see Hänggi, 2002). Nevertheless, several studies have provided evidence for this in the detection of prey (Russell et al., 1999), hydrodynamic turbulence (Douglass et al., 1993), wind generated by a predatory wasp (Levin and Miller, 1996), weak vibratory signals (Spezia et al., 2008) and the reduction in the absolute threshold for the detection of pure tone signals in humans with normal hearing (Zeng et al., 2000). We simulated the active space of pure tone signals in a 2D computer model in order to study the biological relevance of the improved detection of the 2 kHz signal when the trilling species is active at the same time, taking realistic hearing thresholds into account. These simulation results suggest that, under ideal conditions (i.e., sound propagates uniformly in all directions in the absence of background noise), a chirper male that generates a 5 kHz signal and sings at a distance of 19 m remains 1 dB below the hearing threshold of a

conspecific receiver (52 dB SPL). According to the data shown in Fig. 6A, SR is likely to improve signal detection at SNRs between +15 dB and −3 dB, which correspond to simulated triller-receiver distances of 37 m and 10 m, respectively (see Fig. 8). This modelling result suggests a wide range of sender-receiver distances over which the triller male calling song may help to improve the ability of the chirping species to detect subthreshold signals. However, the SR mainly depends on the temporal overlap of signals and loud syllables of the triller song, which is more likely occurr in the field where triller males form choruses in which their songs, but not their syllables, overlap in time (Krobath et al., in press). Nevertheless, the high level of background noise in the habitat may restrict the area within which receivers may benefit from SR. Kostarakos and Römer (2015) made intracellular recordings of several prothoracic interneurons and revealed that their response to a 2 kHz signal was only marginally affected by the high intensities of the heterospecific trill. This result is either the outcome of a tuning to low-frequency sound or the result of strong stimulus-dependent adaptation processes that have taken place in auditory neurons. These neurons are believed to play an important role in the detection of conspecific signals in natural habitats in which the trilling species is also active. In this case, SR may improve the abilities of these neurons to detect signals when the conspecific signaler is singing at a greater distance.

Appendix A. Supplementary data

Refer to Web version on PubMed Central for supplementary material.

Funding

Abbreviations

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SR	Stochastic resonance
TN-1	T-shaped interneuron
SPL	Sound pressure level
SNR	Signal-to-noise ratio
ISI	Interspike intervals

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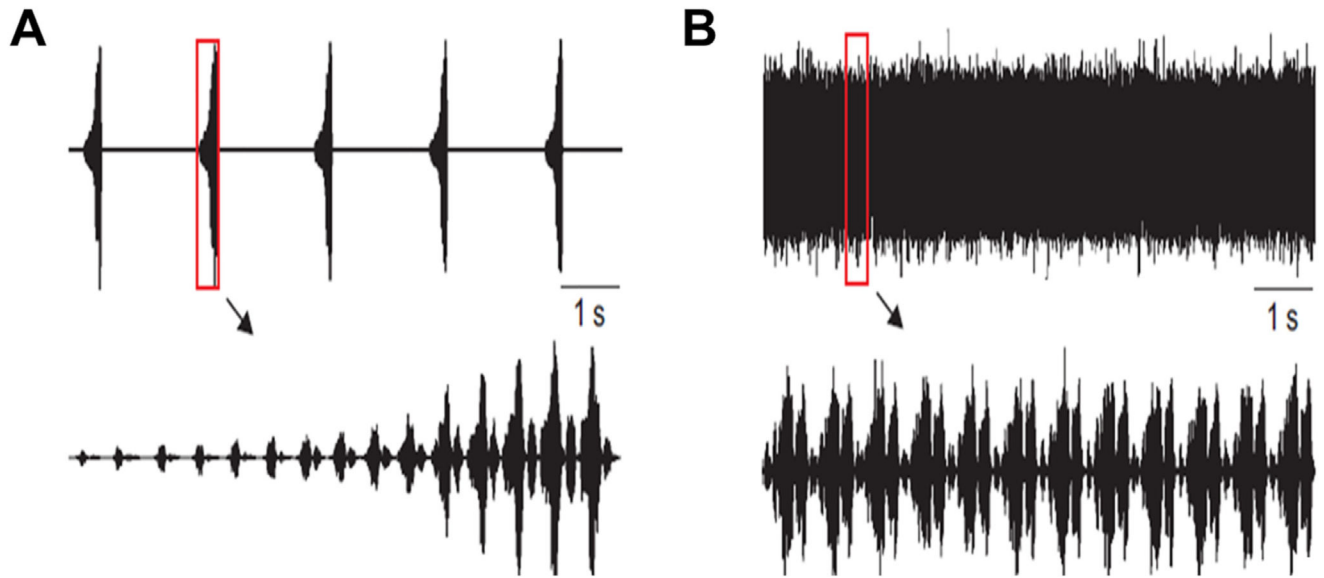
Fig. 1.
Oscillograms of the calling song of the two *Mecopoda* species (reprinted with permission from Siegert et al., 2013). (A) Calling song of the chirping species and the temporal pattern of a single chirp (below). (B) Calling song of the trilling species and the train of syllables magnified below. A two-second part of this trill was used for playback in this study.
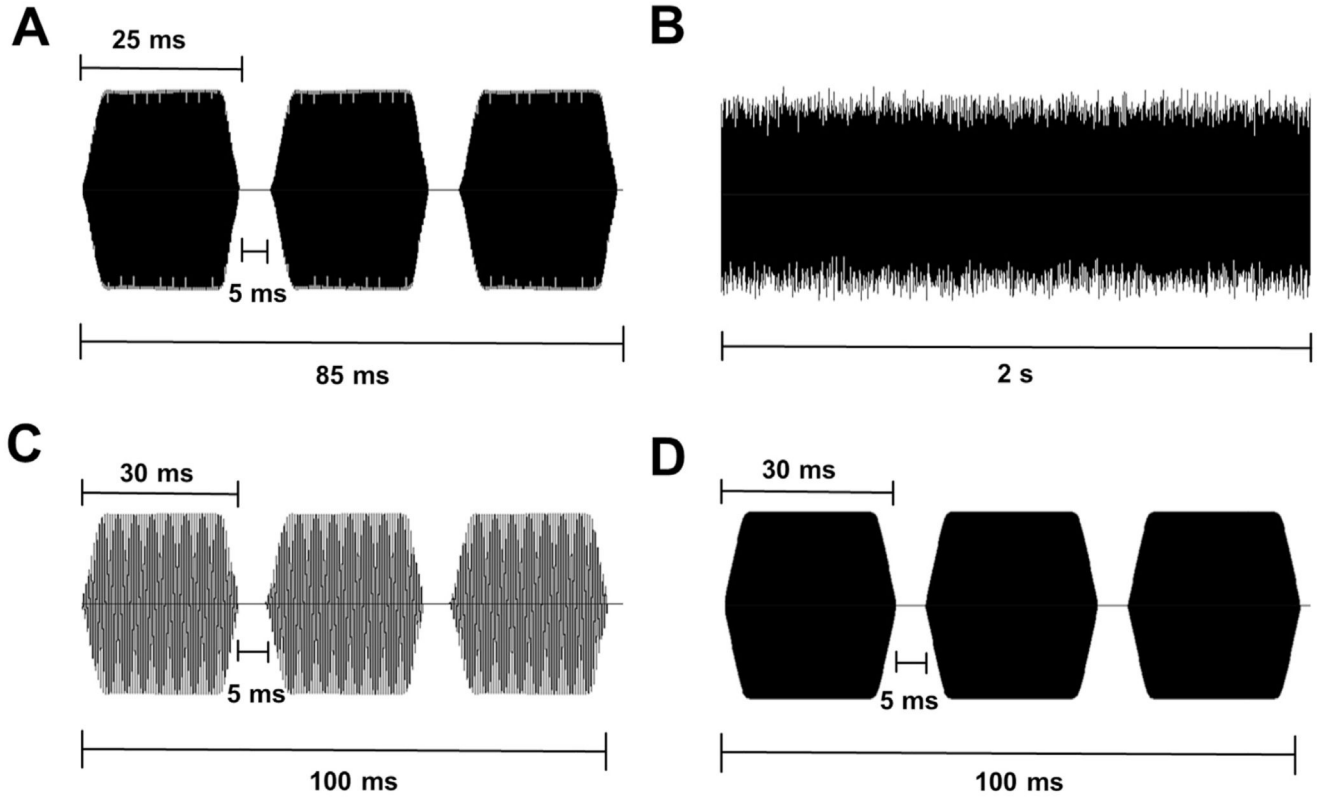
Fig. 2.
Oscillograms of the acoustic stimuli used in SR experiments. (A) Triple-pulsed 20 kHz signal: 25 ms pulse duration and 5 ms pause separating pulses. (B) The white noise signal broadcast in loop mode. (C) Triple-pulsed 2 kHz signal: 30 ms pulse duration and 5 ms pause separating pulses. (D) Triple-pulsed 8 kHz signal: 30 ms pulse duration and 5 ms pause separating pulses.
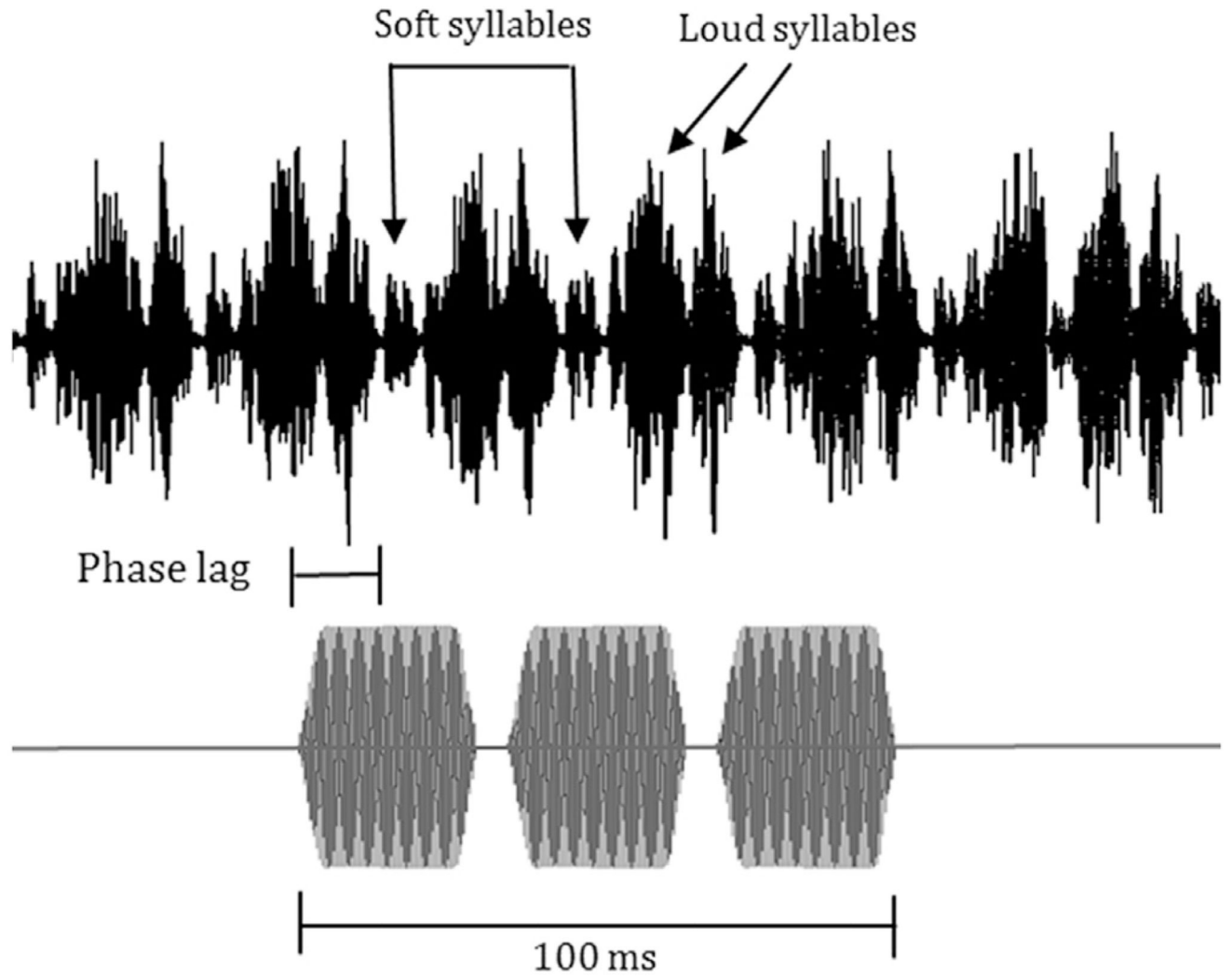
Fig. 3.
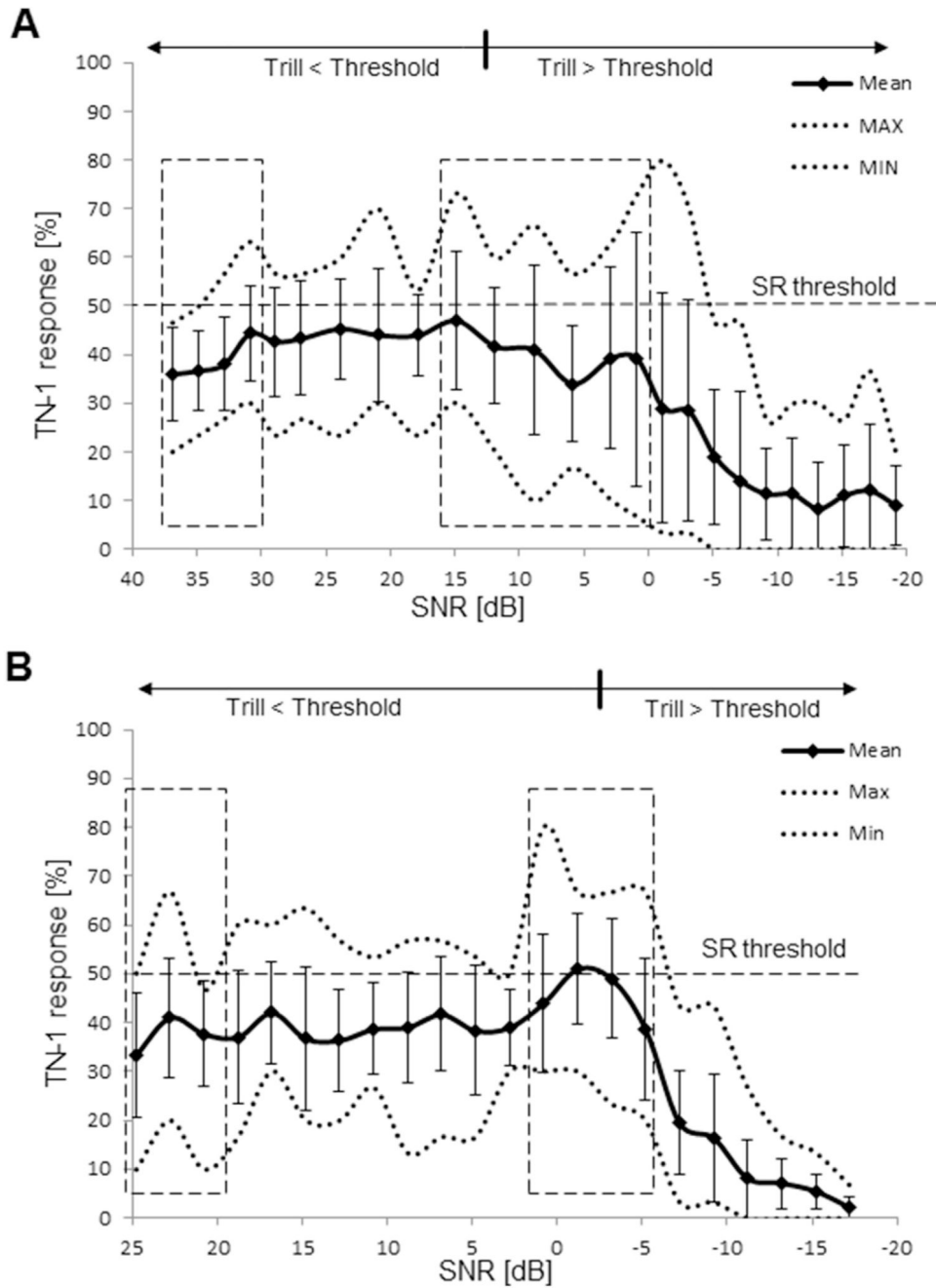Example showing the phase relationship between the 2 kHz signal and the syllables of the trill.
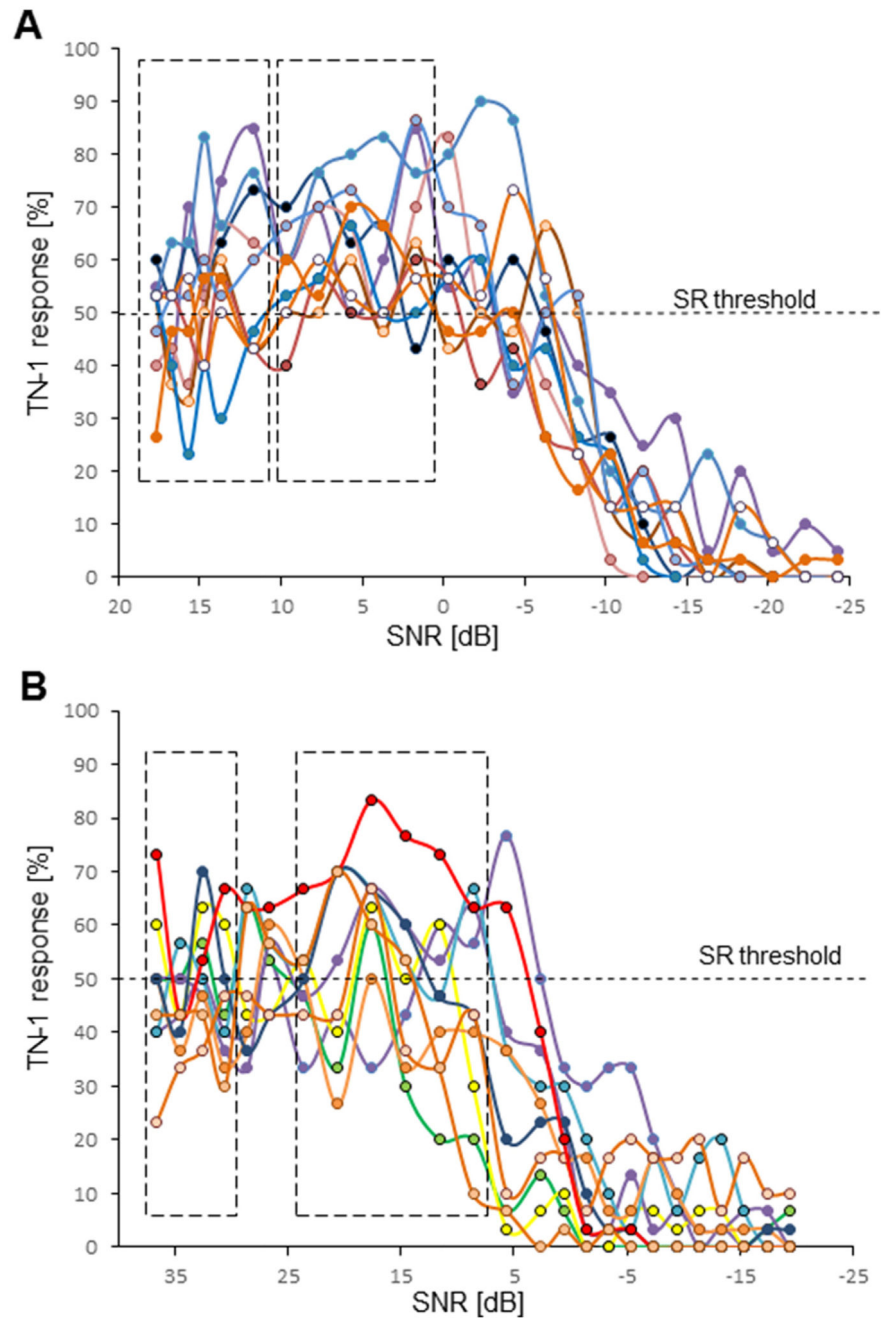
Fig. 4.
Percentage of TN-1 responses to pure tone signals broadcast at a 1 dB subthreshold together with different amplitudes of white noise. (A) 20 kHz signal. (B) 2 kHz signal. Colors in A and B refer to the percentages of TN-1 responses of different individuals. Data in A were obtained from 5 females (orange color) and 5 males (blue or violet colors). Data in B were obtained from 7 males and 3 females (orange color). Dashed boxes refer to the data that were used for comparing the proportions of suprathreshold responses using a z-test. (For

interpretation of the references to colour in this figure legend, the reader should refer to the web version of this article.)
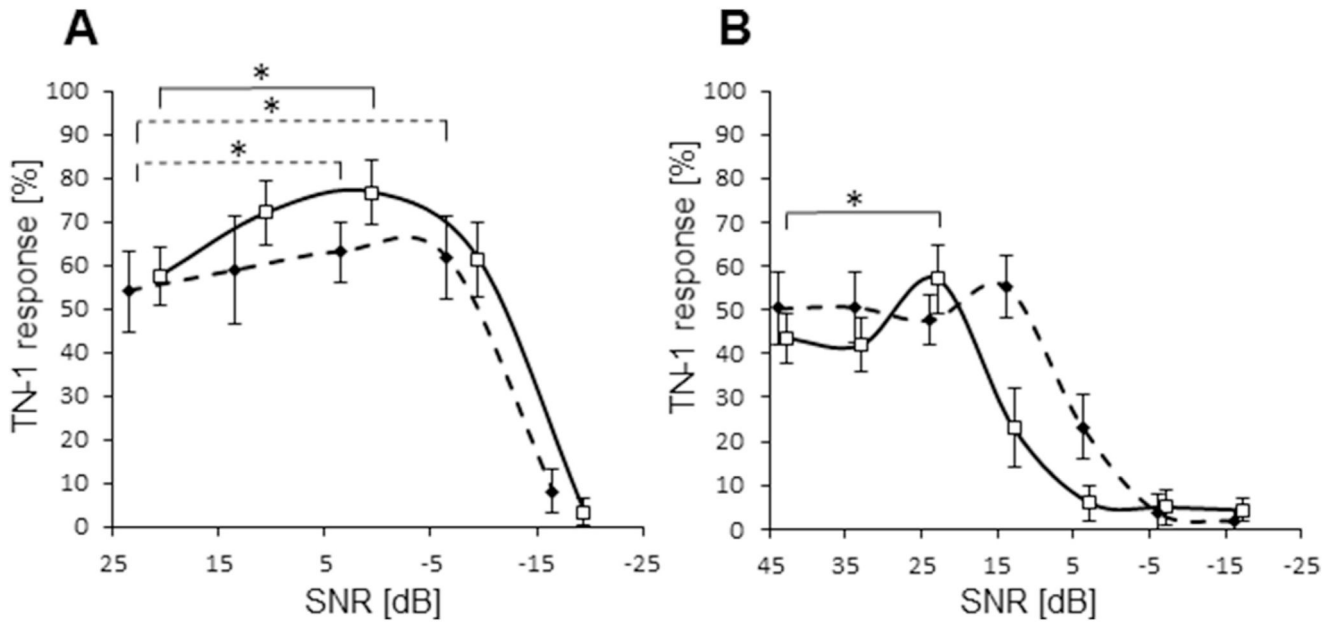
Fig. 5.
The mean percentage of the TN-1 responses of two individuals to repeated presentations of subthreshold pure tone signals at various amplitudes of white noise. Results are based on 10 repetitions of the stimulus sequence at each SNR. (A) 20 kHz signal. (B) 2 kHz signal. Error bars indicate standard deviation. A significant increase in the percentage of TN-1 response is indicated by asterisks ($p < 0.05$, Mann Whitney U test followed by a Tukey post hoc test).

Fig. 6.

The mean percentage of TN-1 responses to the pure tone signals broadcast at a 1 dB subthreshold together with different amplitudes of the trill. (A) 2 kHz signal, mean ± SD of 10 individuals. (B) 8 kHz signal, mean ± SD of 10 individuals. Dashed lines refer to the maximum and minimum values. The vertical tick on top of the figures indicates the mean threshold required to elicit a spiking response to the trill. Dashed boxes refer to the data that were used for comparing the proportions of suprathreshold responses using a z-test.
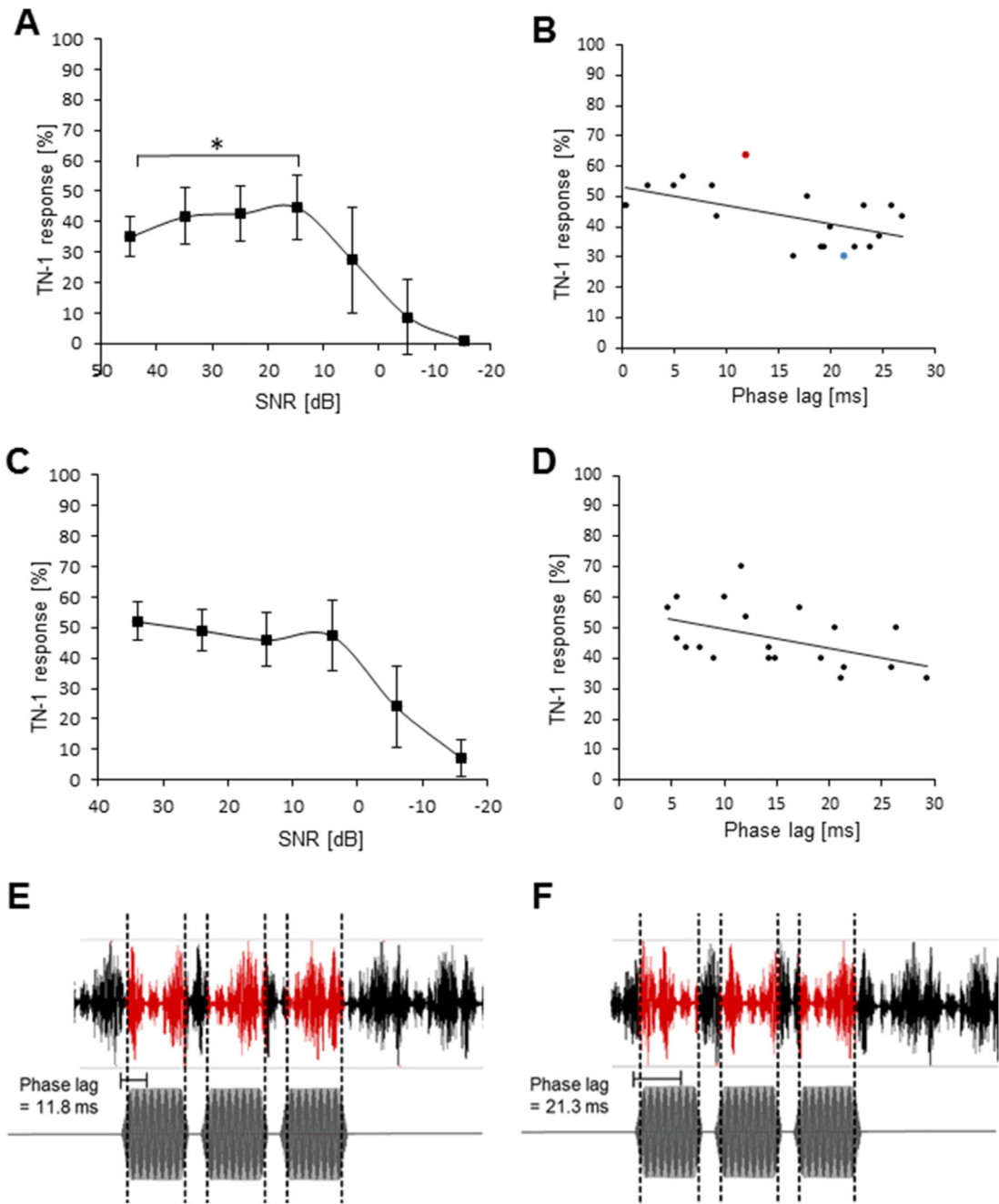
Fig. 7.

Within-individual TN-1 response variability and phase relationships. Mean TN-1 response probability to subthreshold pure-tone signals broadcast at various SNRs together with the trill (A = 2 kHz, C = 8 kHz). Correlation between the spiking probability and stimulus phase lag. (B = 2 kHz; corresponds to +25 and + 15 SNRs, D = 8 kHz; corresponds to +14 and + 4 SNRs). (E) The phase relationship that led to the highest TN-1 response (F) The phase relationship that led to the lowest TN1 response. A significant increase in the percentage of

TN-1 response is indicated by an asterisk ($p < 0.05$, Mann Whitney U test followed by a Tukey post hoc test).
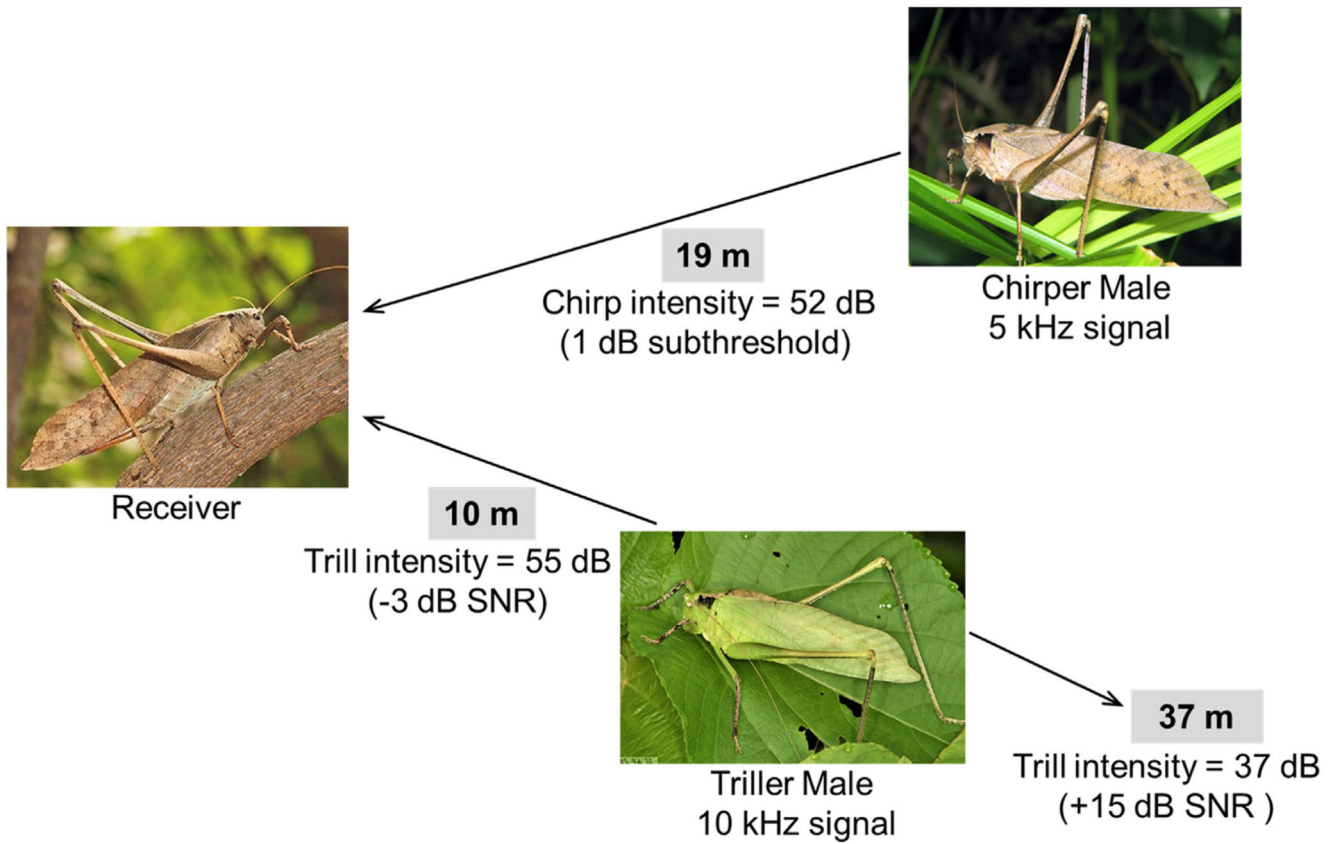
Fig. 8.
Spatial arrangement of signalers and a receiver between which SR is possible. According to the simulation results, the conspecific receiver remained 1 dB subthreshold at a distance of 19 m. When a triller male sings 10–37 m away from the receiver, he can more easily detect the conspecific signaler according to results shown in Fig. 6A. Note that these results neglect the possible influence of other signalers and complex acoustic properties of the natural habitat.