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RESEARCH ARTICLE

Neuronal correlates of a preference for leading signals in the synchronizing bushcricket *Mecopoda elongata* (Orthoptera, Tettigoniidae)

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SUMMARY

Acoustically interacting males of the tropical katydid *Mecopoda elongata* synchronize their chirps imperfectly, so that one male calls consistently earlier in time than the other. In choice situations, females prefer the leader signal, and it has been suggested that a neuronal mechanism based on directional hearing may be responsible for the asymmetric, stronger representation of the leader signal in receivers. Here, we investigated the potential mechanism in a pair of interneurons (TN1 neuron) of the afferent auditory pathway, known for its contralateral inhibitory input in directional hearing. In this interneuron, conspecific signals are reliably encoded under natural conditions, despite high background noise levels. Unilateral presentations of a conspecific chirp elicited a TN1 response where each suprathreshold syllable in the chirp was reliably copied in a phase-locked fashion. Two identical chirps broadcast with a 180 deg spatial separation resulted in a strong suppression of the response to the follower signal, when the time delay was 20 ms or more. Muting the ear on the leader side fully restored the response to the follower signal compared with unilateral controls. Time–intensity trading experiments, in which the disadvantage of the follower signal was traded against higher sound pressure levels, demonstrated the dominating influence of signal timing on the TN1 response, and this was especially pronounced at higher sound levels of the leader. These results support the hypothesis that the female preference for leader signals in *M. elongata* is the outcome of a sensory mechanism that originally evolved for directional hearing.

Key words: auditory neurons, chorus synchrony, contralateral inhibition, rainforest noise, sensory bias.

INTRODUCTION

In aggregations of males, the timing of their mating displays, relative to each other, can vary from completely arbitrary to almost perfect synchrony (reviewed in Greenfield, 1994a; Gerhardt and Huber, 2002). Examples for impressive synchronous displays have been reported for visual signals [fireflies (Buck and Buck, 1968; Buck and Buck, 1978; Lloyd, 1973); fiddler crabs (Backwell et al., 1998)], acoustic signals [anurans (Wells, 1988; Tuttle and Ryan, 1982; Grafe, 1999; Greenfield and Rand, 2000); Orthoptera (Walker, 1969; Sismondo, 1990; Greenfield and Roizen, 1993; Snedden and Greenfield, 1998; Nityananda and Balakrishnan, 2007; Hartbauer et al., 2005; Greenfield and Schul, 2008)] and substrate-borne vibrations [spiders (Kotiaho et al., 2004)]. Therefore, receivers evaluating such displays can base their mating decision on signal timing rather than on other characters (Greenfield, 1994a).

The precise timing of signals is often crucial for the signaller's fitness (reviewed in Gerhardt and Huber, 2002), as females exhibit a preference for the leader of imperfectly synchronized signals in various anuran and insect species (reviewed in Klump and Gerhardt, 1992; Greenfield, 1994b; Grafe, 1996; Grafe, 1999; Snedden and Greenfield, 1998). The preference constitutes a precedence effect, which is defined as the association of a receiver with the leading signal, when two closely timed signals are presented from different directions [humans (Zurek, 1987; Litovsky et al., 1999); mammals, birds, frogs and insects (Cranford, 1982; Wyttenbach and Hoy, 1993; Dent and Dooling, 2004; Lee et al., 2009; Marshall and Gerhardt, 2010)]. A preference for leader signals may have originated from a sensory property in receivers that evolved in a context other than synchronous signalling and has the potential to affect sexual selection (reviewed by Ryan and Keddy-Hector, 1992).

Both 'offset synchrony' and a female preference for the leading signal have been reported for the tropical katydid *Mecopoda elongata.* Males in a chorus within earshot of each other synchronize their signals (chirps) with high precision, causing a high degree of signal overlap (Sismondo, 1990). Different from other synchronizing species, interacting male katydids establish stable leader and follower roles that often persist for a whole song bout (Hartbauer et al., 2005; Hartbauer, 2008). Males that exhibit higher chirp rates while singing in isolation are more likely to become the leader during song interactions with a male singing at a lower solo chirp rate (Hartbauer et al., 2005). *Mecopoda elongata* females exhibited a preference for leader signals in two-choice situations in which identical male chirps were presented with a time delay of 140 ms from separate speakers (Fertschai et al., 2007). The preference for the leader signal is quite strong, as trading experiments in which the advantage of the leader signal was 'traded' against increased loudness of the follower signal revealed values of 10 dB.

In some mating systems, signallers increase the conspicuousness of their displays by exploiting a bias in the processing of sensory information in receivers (Ryan et al., 1990; Ryan and Keddy-Hector, 1992; Endler and Basolo, 1998; Ryan, 1999). In contrast to a mating system in which the signalling trait and preference coevolved, a receiver bias already existed before signallers evolved traits exploiting it (Ryan et al., 1990; Ryan and Rand, 1993; Ryan, 1999).

Römer and colleagues hypothesized that the strong preference for leader signals in *M. elongata* may be the outcome of such a sensory bias in the auditory system of receivers related to directional hearing (Römer et al., 2002).

In katydids, crickets and grasshoppers, interneurons in the auditory pathway have been described that receive excitatory input from the ipsilateral side and strong inhibitory input from the contralateral side (for a review, see Hedwig and Pollack, 2008). Thus, in a stimulus situation with the leader and follower signals presented from opposite sides of the hearing system, the leader initiates an acoustic response in the neuron on this side, but at the same time a strong inhibition in its contralateral, side-homologous counterpart. When the follower signal then starts after some time delay, the existing inhibition suppresses the excitatory action on the follower side. This would result in a strong right–left asymmetry in the response to two otherwise symmetrical signals, and could bias the phonotaxis of females towards the leader signal. Indeed, Römer and colleagues found evidence for such a mechanism in a pair of local interneurons (omega neurons) in the auditory system of katydids (Römer et al., 2002).

However, omega cells are local prothoracic neurons that do not connect to the brain, where decisions are usually made (Stumpner and von Helversen, 2001). Here, we investigated a pair of direction-sensitive TN1 neurons (T-shaped neurons) as a possible neuronal correlate conveying a leader-bias to the brain of a receiver. Although its functional role has been mainly discussed in the context of detection of bat-like ultrasound and corresponding avoidance behaviour (Faure and Hoy, 2000c; Schul et al., 2000; ter Hofstede et al., 2010), Schul made a strong point for its involvement in coding conspecific signals (Schul, 1997). The tuning of the neuron (broadband, including ultrasonics in the *Mecopoda* calls) as well as the strong contralateral inhibition (McKay, 1969; Schul, 1997; Faure and Hoy, 2000b; Rheinlaender and Römer, 1980; Suga and Katsuki, 1961; Rheinlaender et al., 1972) are prerequisites for its role in selectively encoding leader signals.

Here, we investigated the response of both TN1 neurons simultaneously in stimulus situations with identical male signals differing in signal onset timing and loudness. The TN1 response was also investigated in playback experiments in which conspecific signals were broadcast in the presence of nocturnal tropical rainforest noise, as such background noise may impair the representation of leader and follower signals in the CNS of receivers.

MATERIALS AND METHODS

Insects

Male and female *M. elongata* L. (Orthoptera, Tettigoniidae, Mecopodinae) were taken from a laboratory breed originally established with individuals collected in the tropical rainforest in Malaysia (Ulu Gombak, Selangor, Kuala Lumpur). Insects were reared in colonies at a temperature of 27°C, 70% relative humidity, on a 12 h:12 h light:dark schedule. They were fed *ad libitum* with fish food, oat flakes and fresh lettuce. In the genus *Mecopoda* there are several sibling species that can be distinguished by their morphology, but are more easily differentiated by their song pattern. In the breeding population, males produce songs identical to the songs of 'species S' described by Sismondo (Sismondo, 1990) with a mean chirp period of 2 s.

Neurophysiology

Bilateral extracellular recordings were made from axons of the TN1 neuron (Suga and Katsuki, 1961; McKay, 1969). Soma and input synapses with auditory receptor projections of this neuron are located in the prothoracic ganglion; an ascending axon on the soma-contralateral side runs through the connectives to the suboesophageal and supraoesophageal ganglion and terminates in the brain. We have not explicitly shown for *M. elongata* (by virtue of intracellular recording and staining) that the large action potentials (APs) in extracellular recordings are from the cell known as TN1. However, in another katydid (*Platycleis affinis*) an experiment with one intracellular electrode in the dendrites and a pair of hook electrodes recording simultaneously from the cervical connectives confirmed the identity of intracellular and extracellular APs (Rheinlaender, 1984). As the cell was later stained and morphologically identified as TN1, we are confident that the only large spike activity in response to sound stems from TN1. Further, in many tettigoniid species this neuron has been shown to be the only afferent auditory neuron generating large amplitude spikes using the hook electrode recording technique (Schul, 1997; Faure and Hoy, 2000b; ter Hofstede et al., 2010). Methodological details for bilaterally recording the activity of the pair of TN1 neurons are described elsewhere (Rheinlaender and Römer, 1980; Faure and Hoy, 2000a). In brief, both cervical connectives were surgically exposed and separately hooked by a pair of tungsten electrodes. The preparation was placed ventral side up in an anechoic chamber between two speakers separated from the preparation by 50 cm. Neuronal responses were amplified using a custom-made biosignal amplifier (Land et al., 2001) and digitized at 20 kHz in Chart V5.5.6 (AD Instruments, Spechbach, Germany) using a PowerLab multi-channel recorder (AD Instruments).

Acoustic stimulation

The calling song of a male singing in isolation is a series of regularly repeated chirps (chirp periods range between 1.6 and 2.3 s; mean 2.0 s). Each chirp lasts for 200–300 ms and consists of individual syllables increasing in amplitude (Hartbauer et al., 2005). For playbacks, a representative chirp with a syllable rate of 55 Hz and duration of 270 ms was used. This chirp was recorded from a male singing in isolation and is hereafter termed 'solo signal'. In addition, a sound recording of three males signalling in synchrony was used as a 'chorus signal'. The chorus signal lacked a distinct syllable structure, lasted for 300 ms and contained less energy at ultrasonic frequencies above 20 kHz (Fig. 1). All sound recordings were performed at a distance of about 20–30 cm from singing males in an anechoic chamber using a 1/4 in microphone (type 2540, Larson Davis, Depew, NY, USA) mounted to a sound level meter (CEL 414, Casella, Bedford, UK). The sampling rate during sound recordings was 96 kHz.

Different leader–follower situations in which leader and follower signals exhibited a certain time delay were created by shifting identical sound signals in time using sound editing software (Cool Edit Pro 2.0, Syntrillium Software, Phoenix, AZ, USA). Leader and follower signals were broadcast *via* two speakers from opposite sides of the insect preparation (Leaf tweeter, Technics EAS-10TH400A, Kadoma, Japan). Signal period was fixed to 2 s in order to mimic the natural chirp period of the species. Signals were broadcast in continuous loops from two output channels of a D/A firewire sound card (Edirol FA-101, Roland Inc., Tokyo, Japan). After passing a linear attenuator (PA-5, Tucker Davis Inc., Alachua, FL, USA) signals were amplified by a stereo amplifier (NAD 214, NAD Electronics, Pickering, ON, Canada). The sound pressure level (SPL) was calibrated relative to 20 μPa at the position of the preparation by continuous playback of only the last syllable within the chirp (exhibiting the maximum amplitude). For calibration, a condenser microphone with a flat frequency response characteristic between

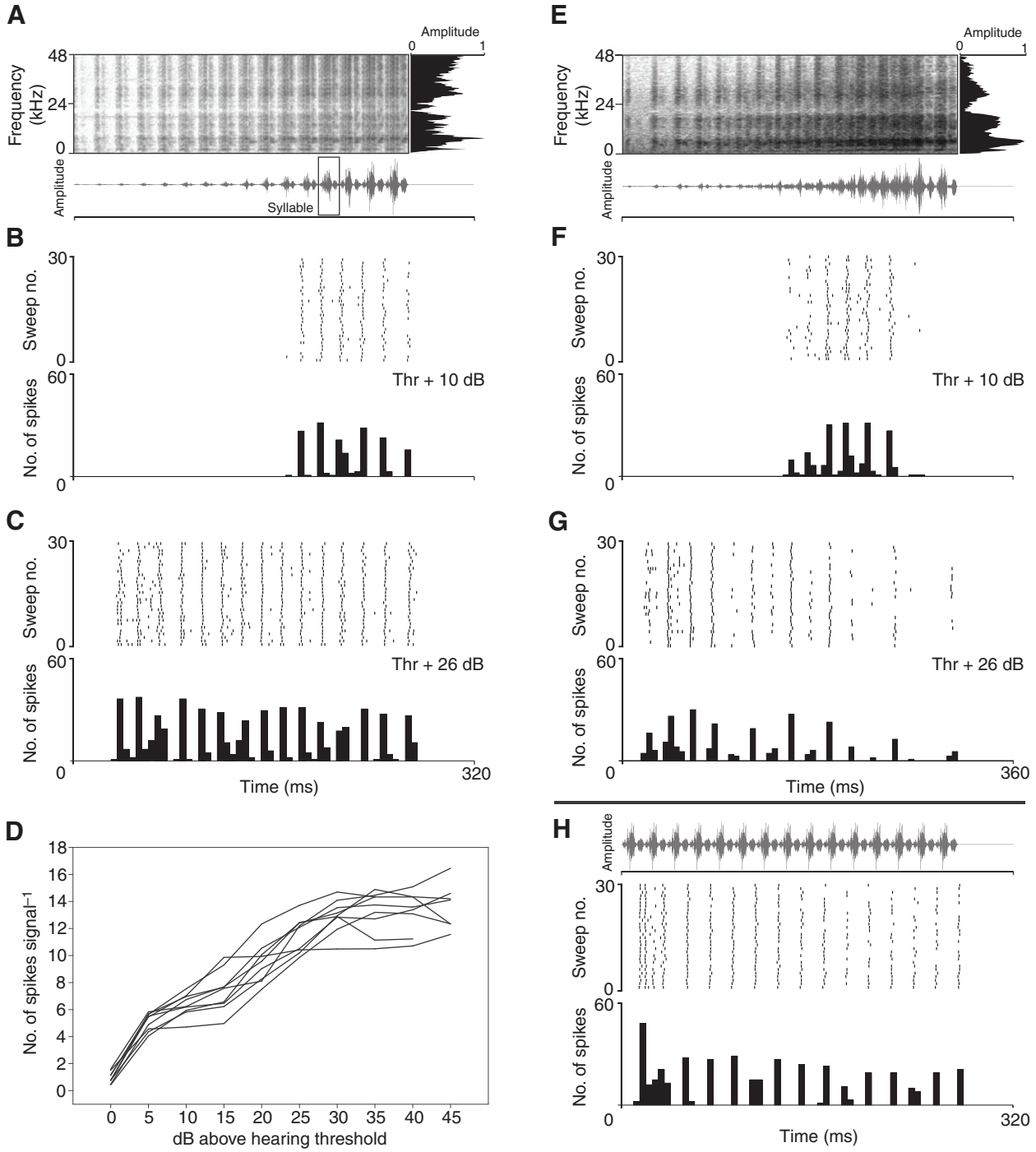

Fig. 1. TN1 response to conspecific signals used for playbacks. (A) Sonogram and oscillogram (below) of a *Mecopoda elongata* chirp termed a 'solo signal' in playback experiments. Chirps consist of syllables increasing in loudness, where each loud hemi-syllable is followed by a soft hemi-syllable (indicated by a rectangle). The TN1 response to the solo signal broadcast at 10 dB (B) and 26 dB (C) above threshold (Thr) is shown as peri-stimulus time histogram (PSTH) and raster plot. (D) Intensity–response functions of nine TN1 preparations in response to the solo signal (mean hearing threshold, 38 dB sound pressure level, SPL). (E) Sonogram and oscillogram (below) of a conspecific signal recorded next to three imperfectly synchronized males ('chorus signal'); it lacks a clear syllable structure. (F,G) TN1 responses to the chorus signal presented at 10 dB (F) or 26 dB (G) above hearing threshold. (H) TN1 response to an artificial signal consisting of '*Mecopoda* syllables' equal in loudness (65 dB SPL; syllable interval, 17 ms). Bin size of PSTHs is 5 ms.

4 Hz to 48 kHz was used (LD 2540, Type 4133, Larson Davis). Calibration was carried out in 'fast' reading mode with the sound-level meter CEL 414 attached to a filter unit (CEL-296). Both types of signal were calibrated to a peak SPL of 65 dB at the position of the insect preparation.

Experiments with environmental noise

In order to study the encoding of the 'solo signal' in the presence of natural background noise, a continuous playback of a 70 s segment of noise was calibrated to a SPL of 65 dB, whereas the SPL of the solo signal was systematically varied to achieve

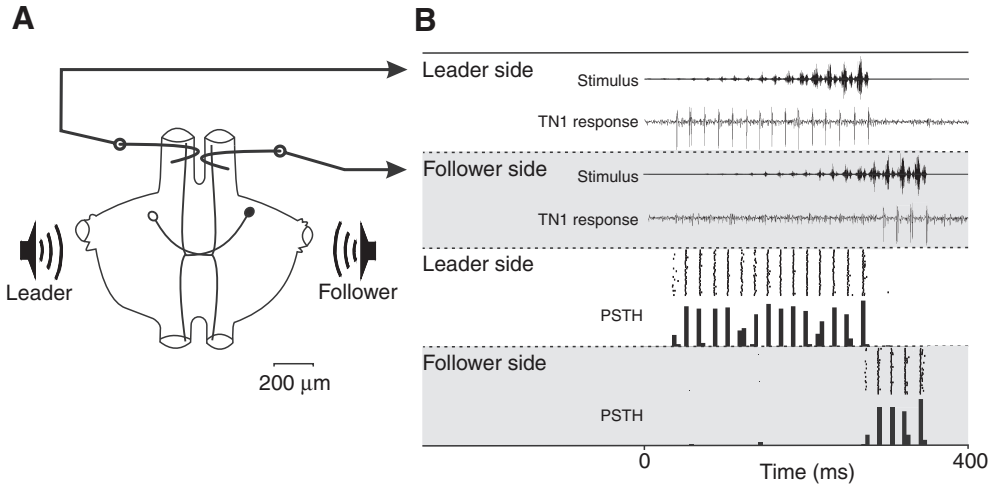

Fig. 2. Arrangement of bilateral TN1 recording sites and stimulation in the leader–follower situation. (A) Sketch of the gross anatomy of paired TN1 neurons in the prothoracic ganglion and position of a pair of hook electrodes for recording TN1 discharges in the neck connective. (B) Bilateral TN1 response to a conspecific solo chirp presented from opposite sides with a time delay of 70 ms at 65 dB SPL. Note the strong asymmetrical discharge of the two neurons.

different signal-to-noise ratios. The background noise had originally been recorded in the understory of a nocturnal rainforest on Barro Colorado Island (Panama) and includes various heterospecific signals of insects, frogs and vertebrates in the audio and ultrasonic frequency range (see also Hartbauer et al., 2010). A dominant frequency band in nocturnal rainforest noise between 4 and 9 kHz is generated by different cricket species occupying narrow frequency bands (Riede, 1997; Ellinger and Hödl, 2003). In addition to this ongoing sonic noise, transient high-frequency signals as well as ultrasound contribute to nocturnal rainforest noise (Balakrishanan, 2005; Hartbauer et al., 2010). We did not use the original Malaysian noise as a background stimulus as in virtually all nocturnal rainforest recordings one or both of the *Mecopoda* species (either the chirper or trilling species) were singing. Therefore, these recordings could not be used as background when systematic playbacks in the lab should reveal the extent of responses to the conspecific stimulus. However, the recording of Panamanian rainforest noise had a rather similar spectral content and similar variants of heterospecific signals compared with Malaysian background noise recordings (compare also sonograms) (Riede, 1997; Ellinger and Hödl, 2003; Balakrishnan, 2005; Lang et al., 2005; Hartbauer et al., 2010).

In the present study, rainforest noise was either broadcast as complete spectrum noise covering the frequency range between 2 and 30 kHz, or as low-pass sound after filtering, which removed all signals beyond 9 kHz. This approach allows a separate investigation of the masking effect arising from calling songs of crickets and katydids. The conspecific chirp signal and the background noise were broadcast *via* two different loudspeakers (Leaf tweeter, Technics EAS-10TH400A) to the ipsilateral side of the TN1 preparation.

In addition to these playback experiments, we investigated the coding of conspecific chirps in a natural setting, using a sound recording of a single male obtained in the field (distance 3 m; next to the field station in Ulu Gombak). This sound recording was used as a playback signal, because it comprises both the conspecific signal and the background noise for a receiver at this distance.

Data analysis

Sonograms of sound signals were calculated in Spike 2 (v5.2.1, Cambridge Electronic Design, Cambridge, UK) using a Hanning window function and a fast Fourier transform (FFT) size of 512 points. Bilateral neuronal recordings were evaluated by means of a custom-written Spike2 macro.

Statistics

Statistics were calculated in Sigma Plot 11.0 (Systat Software Inc., Chicago, IL, USA). Individual groups were tested for significant differences using a Mann–Whitney rank sum test. All significance values were Bonferroni corrected in order to account for multiple testing of single individuals.

RESULTS

TN1 response in single speaker experiments

The solo signal evoked a characteristic neuronal discharge in the TN1 neuron ipsilateral to the active speaker. When the peak amplitude of the signal exceeded the hearing threshold by only 10 dB, the response was restricted to the most intense syllables at the end of chirps (Fig. 1B), whereas higher intensities (+16 dB) elicited a stronger response because the soft syllables marking the onset of chirps became suprathreshold (Fig. 1C). The neuronal response revealed a high temporal precision owing to a reliable encoding of every syllable by a single spike. Although these syllables strongly increase in amplitude towards the end of a chirp, the neuronal response to each suprathreshold syllable was similar. Intensity response functions obtained from different individuals showed a linear increase of TN1 spike count up to a sound level of 30 dB above hearing threshold, where response strength saturated (Fig. 1D).

The chorus signal broadcast at 10 dB above threshold resulted in a response similar to that to the solo signal (compare Fig. 1B,F and Fig. 1C,G). However, higher intensities resulted in some spike adaptation so that the final synchronized chirp elicited little to no response (Fig. 1F,G). An artificial signal consisting of a sequence of '*Mecopoda* syllables' all broadcast at the same peak amplitude (26 dB above hearing threshold) elicited a strong TN1 discharge and a reliable phase-locked spiking to each of the following syllables (Fig. 1H).

TN1 response to leader–follower signals differing in onset timing

Acoustic interactions between males often result in stable leader–follower relationships in which one male consistently leads the other (Hartbauer et al., 2005). Here, we mimicked such stable leader–follower relationships by broadcasting identical chirps with a fixed time delay from opposite sides of a receiver, while simultaneously recording the activity of both TN1 neurons (Fig. 2A). In the example shown in Fig. 2B, a time delay of 70 ms between the two solo signals resulted in a strong leader-biased TN1 response

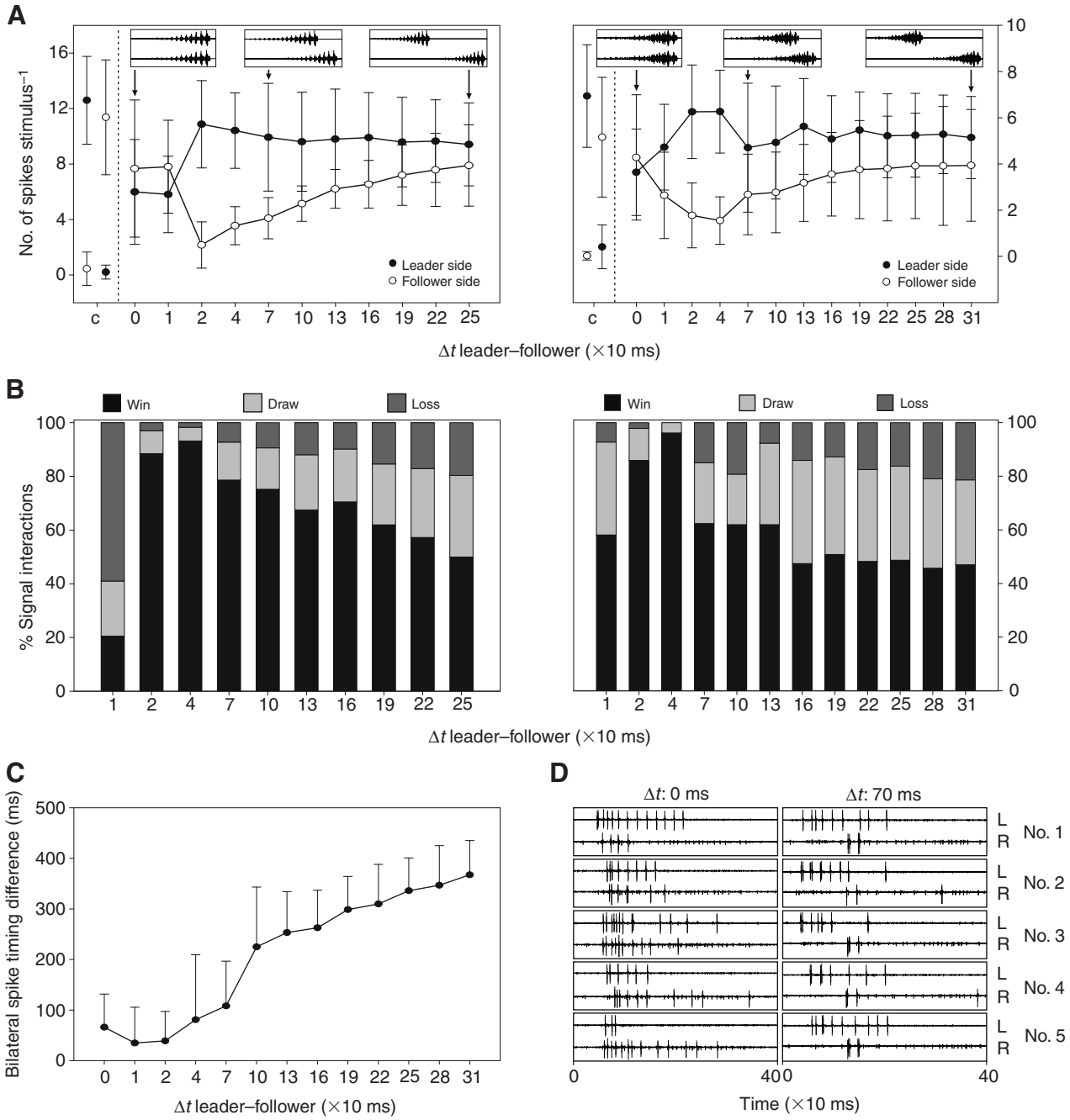

Fig. 3. Binaural responses of the pair of TN1 neurons at various time delays. Identical solo signals (left panels) and chorus signals (right panels) presented from opposite sides at various time delays. (A) Mean bilateral TN1 response at various leader–follower delays. c, unilateral controls. (B) Chirp-by-chirp evaluation of bilateral TN1 responses (see Results for further explanations). (C) Bilateral time difference in the onset of TN1 responses at various time delays. Data in A–C represent the mean of 234 stimulus presentations; 10 preparations. (D) Five examples of simultaneously recorded responses to the chorus signal presented either simultaneously (Δt=0) or at a time delay of 70 ms (leader signal on the left side). L, left; R, right. Error bars indicate s.d. SPL of all signals was 65 dB SPL. Note that the *x*-axis in A–C is not linear.

due to a strong suppression of the response to the follower signal restricting APs to the last syllables of the chirp. As a result, the 70 ms time delay in the onset of leader and follower signals is amplified 2.3 times in the bilateral representation of the signals (100 *vs* 230 ms). Broadcasting leader and follower signals from the same, ipsilateral side resulted in an almost perfect copy of the syllable pattern of both signals in the TN1 response. However, during signal overlap it is almost impossible to determine which signal elicited a given AP.

The leader-biased TN1 response was further investigated by systematically shifting the time delay between leader and follower signals (Δt). Fig. 3A shows the average effect on the discharge of a pair of TN1 neurons (10 preparations, 234 stimulus repetitions). Time delays of 20–190 ms between leader and follower signals resulted in a significantly higher spike count ipsilateral to the leader side (P<0.01; Mann–Whitney rank sum test). When the delay of solo signals differed by 0 and 10 ms the response strength of the two TN1 neurons was similar, but clearly reduced compared with

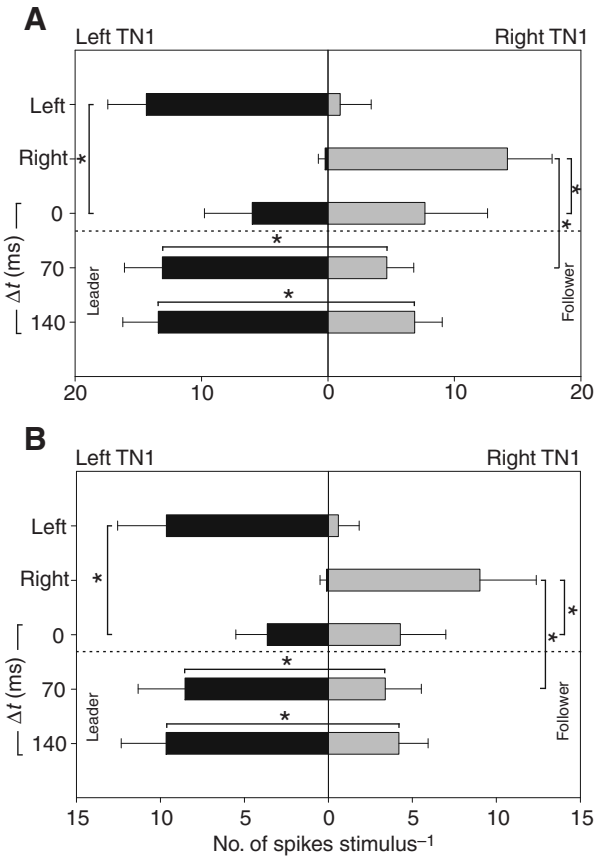

Fig. 4. Summary of bilateral TN1 responses under three different delays of leader–follower presentations. Mean bilateral TN1 response of 9 individuals to either the solo signal (A) or the chorus signal (B) at delays of 0, 70 and 140 ms. Note the significant reduction in response when the two signals were presented at the same time (P<0.01). Each leader–follower situation was presented 30 times. SPL of all signals was 65 dB SPL. Error bars indicate s.d. In this and the following figures, asterisk denotes a significant difference between groups (P<0.01, Mann–Whitney rank sum test).

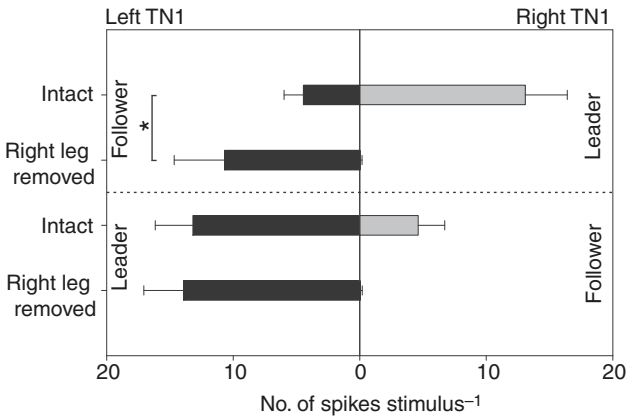

Fig. 5. The source of suppression of the follower signal in TN1 responses. Representation of the leader and follower signals in eight intact preparations (solo signal at 65 dB SPL; time delay 70 ms; upper values) and after removing the sensory input on either the leader side or the follower side. Note that only removal of the input ipsilateral to the leader side resulted in a significant increase in response to the follower (P<0.01). Thirty stimulus presentations for each data point. Error bars indicate s.d.

controls. Even when the two signals did not overlap in time (Δt=250 ms) the response to the leader was significantly increased compared with the response elicited by the follower. A comparison of the mean excess spike count on the leader side showed no sex-related difference in the mean leader-biased response of TN1 [Δt=70 ms, 65 dB SPL: 8.3±1.5 (males) *vs* 8.7±3.5 (females), P=0.50, Mann–Whitney rank sum test, 8 males and 14 females]. Delay variations performed with the chorus signal resulted in a similar asymmetrical representation in favour of the leader signal, starting with delays of 10 ms (Fig. 3A, right; P<0.01; Mann–Whitney rank sum test).

Because receivers may base their decisions on a single stimulus presentation, rather than averaging over many stimuli, we additionally analysed the bilateral representations of the leader and follower signal on a chirp-by-chirp basis. A higher spike count on the leader side was considered a 'win', the opposite situation a 'loss'. Equal spike counts (±1 spike stimulus^{-1}) were considered a 'draw'. This kind of evaluation revealed that a win situation clearly dominated for the leader when solo signals had a delay of more than 20 ms (Fig. 3B, left). The same was true for chorus signal delays of between 20 and 130 ms (Fig. 3B, right), but Δt values of more than 130 ms reduced win situations to about 50%.

As already evident in the recording example of Fig. 2, a leader–follower delay of 70 ms resulted in latency differences of their respective responses that were much larger than the physical time delays of the stimuli. We therefore analysed these latency differences in addition to the response strength (Fig. 3C). A time delay of 40 ms in the onset of solo signals delayed the response on the follower side by almost 100 ms compared with that on the leader side. A delay of 100 ms delayed the response to the follower by 220 ms.

The strong impact of the temporal advantage of the leader on the latency differences of responses is also demonstrated in single stimulus representations in Fig. 3D. In all five repetitions with a temporal advantage of 70 ms, the neuron on the leader side fired more strongly (about eight APs compared with three APs), but the latency differences were more than 1.4-fold relative to physical time differences. Without such a temporal advantage, each auditory side receives the same stimulus at exactly the same time (Fig. 3D, left). Interestingly, under such symmetrical stimulus situations the individual responses of the two neurons are rarely symmetrical. Rather, either the left or the right neuron fires more strongly, and the neuron dominating the response switches randomly between sides.

The mean bilateral TN1 response for time delays of 0, 70 and 140 ms for both types of signal is summarized in Fig. 4. The solo signal presented alone to either side elicited almost exclusively a response in the ipsilateral neuron (Fig. 4A, top panels). A simultaneous presentation of these signals from opposite sides reduced the response significantly compared with unilateral stimulation (P<0.01, Mann–Whitney rank sum test, N=270), but on average the two responses were similar. In contrast, time delays of 70 and 140 ms significantly reduced the representation of the follower signal. This is obvious by comparing either the unilateral response with the leader–follower response obtained at the two delay values, or the responses to leader and follower signals directly. Notably, bilateral stimulation did not affect the representation of the leader signal compared with unilateral stimulation. Switching leader and follower signals between the two sides reversed the leader-biased response in both neurons (data not shown).

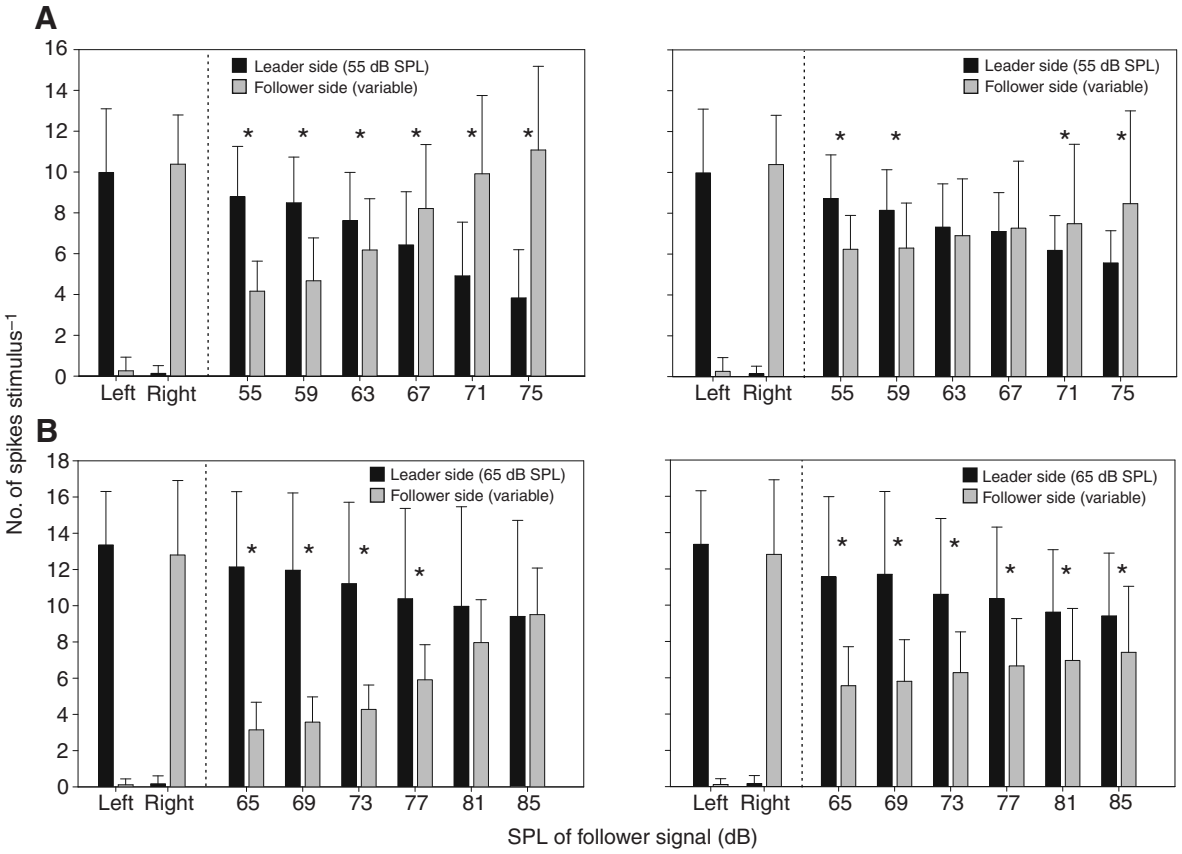

Fig. 6. Time–intensity trading experiments. The SPL of the follower signal was successively increased relative to that of the leader at delays of 70 ms (left panels) and 140 ms (right panels); leader signal at 55 dB SPL (A) or 65 dB SPL (B). Note that the temporal disadvantage of the follower signal in the bilateral TN1 representation can be compensated for with an increase in follower loudness, except at a delay of 140 ms and a leader signal SPL of 65 dB. *N*=5 preparations, 30 stimulus presentations for each data point. 'Left' and 'Right' refer to responses with unilateral stimulation.

The source of the strong suppression of TN1 responses to follower signals was demonstrated using a comparison between binaural and monaural preparations. In a leader–follower situation (solo signal; time delay 70 ms) the removal of the sensory input ipsilateral to the leader signal resulted in a significant increase of the response to the follower signal (upper panel of Fig. 5, mean of eight preparations). By contrast, removal of input ipsilateral to the follower signal did not change the response to the leader signal, but of course completely abolished the response to the follower signal of the opposite TN1 neuron.

Time–intensity trading

Previous experiments show that the advantage of the leader signal in its representation in the afferent auditory pathway can be compensated for (traded) by increasing the loudness of the follower signal (Fertschai et al., 2007). Here, we performed time–intensity trading experiments in two situations of leader–follower interactions, at time delays of either 70 or 140 ms (Fig. 6) using two different sound levels of the leader signal (55 and 65 dB SPL). When the two solo signals were broadcast at equal intensity a temporal advantage of 70 ms resulted in a strong leader-biased asymmetry of the respective responses. An increase of the SPL of the follower signal decreased the leader-biased response, which finally disappeared at a follower SPL of 81 dB and a leader SPL of 65 dB (Fig. 6B left). This reversal of bilateral excitation was found at 67 dB for a leader SPL of 55 dB (Fig. 6A, left). The same trading experiment using a time delay of 140 ms (Fig. 6A, right) yielded similar results. However, trading the biased responses against an increased loudness of the follower had little effect in experiments broadcasting the leader signal at 65 dB SPL. Here, even an advantage of 20 dB for the follower was unable to compensate for the timing advantage in favour of the leader signal (Fig. 6B, right).

TN1 response in the presence of rainforest noise

The natural habitat of *M. elongata* is the tropical rainforest of Malaysia, and nocturnal rainforests are well known for their high background noise levels (Lang et al., 2005; Riede, 1997; Ellinger and Hödl, 2003; Hartbauer et al., 2010). We therefore investigated the amount of masking of conspecific signals at realistic levels of rainforest background noise (Fig. 7A). In these experiments either full spectrum noise (<30 kHz) or low-pass filtered noise (<9 kHz) was presented together with the solo signal, *via* separate speakers from the same, ipsilateral side. The full spectrum background noise at a mean SPL of 65 dB reduced the TN1 response to the solo signal strongly compared with control experiments presenting the solo signal without noise (C1 in Fig. 7A). A successive increase of the SPL of the solo signal slightly increased the TN1 response to the signal, but even a signal-to-noise ratio of +10 dB was unable to fully restore the response compared with the control. On average, full spectrum noise more effectively suppressed the TN1 response than did 9 kHz low-pass filtered noise (Fig. 7A). Interestingly, almost no TN1 activity was elicited in response to either noise presented in

loop mode, although spikes were counted in the same time segments for the evaluation of solo signals (C2 in Fig. 7A). Thus, although responses of TN1 are strongly reduced in the presence of rainforest noise, a selective encoding of the conspecific signal was maintained.

This result was confirmed in playback experiments using a sound that was originally recorded in a clearing of the tropical rainforest (Ulu Gombak), at a distance of 3 m from a singing *M. elongata* male. A segment of this sound recording including two successive chirps and moderate background noise (Fig. 7B) was broadcast at a SPL of 65 to TN1 preparations in loop mode. The TN1 response to this sound revealed a selective encoding of the most intense syllables at the end of chirps without a single AP elicited in response to any kind of sound present in the natural background noise (Fig. 7B).

In addition, we quantified the shift in hearing threshold for pure tones in the presence of native rainforest noise broadcast at 65 dB SPL (Fig. 8). This noise resulted in an increase in hearing threshold for pure tones in the frequency range between 5 and 25 kHz. The largest threshold shift (17 dB) was found at 15 kHz (mean of five preparations).

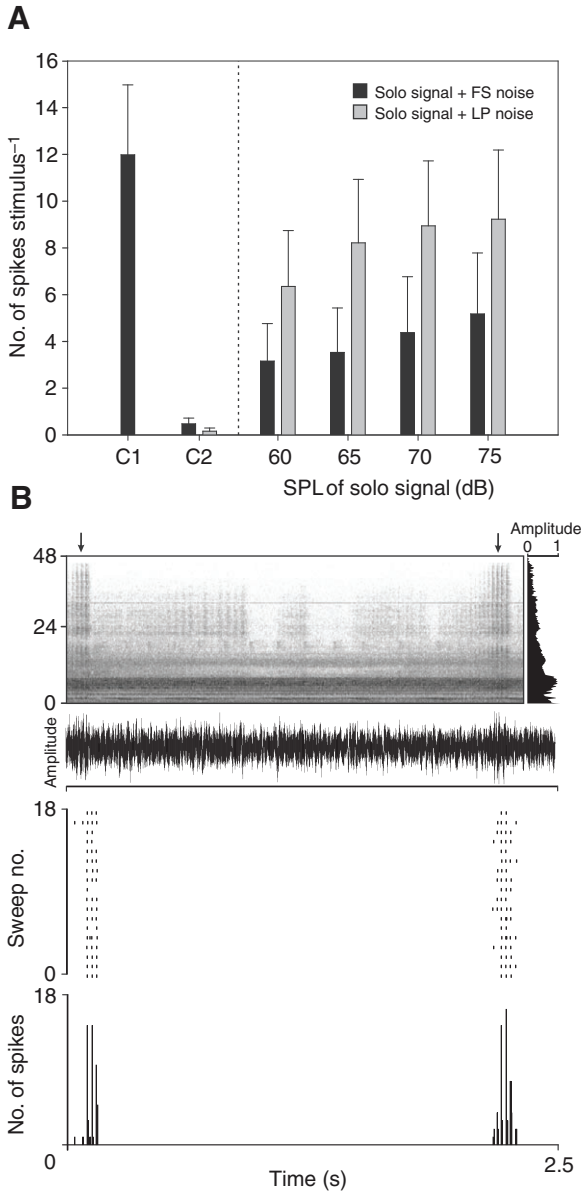

Fig. 7. Signal representation under masking background noise. TN1 response to increasing levels of solo signals studied together with either full spectrum (FS) rainforest noise or a low-pass filtered sonic frequency band (LP). The SPL of noise was always 65 dB. Note the strong reduction in the TN1 response, in particular with full spectrum noise. C1, controls without background noise; C2, response to rainforest noise alone. *N*=8 preparations; mean ± s.d. of 240 stimulus presentations. (B) Upper graph, sonogram (above) and oscillogram (below) of a sound recording obtained in the nocturnal Malaysian rainforest, with two successive chirps of a *M. elongata* male (arrows). Lower graph, PSTH and raster plot of the ipsilateral TN1 response to this sound at 65 dB SPL in loop mode. Note the exclusive TN1 response to the conspecific chirps despite the presence of other HF sounds in the playback. Bin size of PSTH=5 ms.

DISCUSSION

As for other species of Tettigoniidae, *M. elongata* males sing in choruses, where their chirps are organized in either synchronous or (less likely) alternating bouts (Sismondo, 1990; Hartbauer et al., 2005). The synchrony in these choruses is not perfect, however, so that longer sequences of interaction can occur where the chirps of males overlap in time, but one male (the leader) calls consistently earlier than his opponent (the follower) (Hartbauer et al., 2005). Females, when given a choice between spatially separated leader and follower male signals, prefer the leader in *Mecopoda* and other species of Tettigoniidae (Greenfield and Roizen, 1993; Snedden and Greenfield, 1998; Fertschai et al., 2007).

Do female preferences for leading chirps result from a sensory bias?

Remarkably, the preference is not due to some physical masking of the follower's signal by the leader, but appears to result from the precedence effect associated with directional hearing, where the first incoming signal inhibits the response to the follower signal. Because of the potential involvement of directional hearing in the female preference, Römer and colleagues (Römer et al., 2002) proposed that leader males might exploit an existing bias in the nervous system of receivers (*sensu* Ryan et al., 1990; Ryan, 1999). The sensory bias hypothesis assumes that preferences of females originally evolved in a context different from the actual one, or as an epiphenomenon of general sensory mechanisms, which subsequently were exploited by males. Thus, in a receiver bias model the female preference exists before the male trait evolves. As in vertebrates, contralateral inhibition plays a key role in directional hearing and sound localization in insects, by enhancing bilateral peripheral differences (reviewed in Hedwig and Pollack, 2008). Such a mechanism would represent a very general processing mechanism in all animals equipped with two ears. Indeed, the analysis of the responses of a pair of direction-selective interneurons in *M. elongata* (omega neurons) in a leader–follower situation revealed a strong asymmetric representation of two identical signals differing in onset timing (Römer et al., 2002).

We observed no difference in the precedence effect in male and female preparations, which could indicate that the effect is based on a more general, perhaps non-sexual, mechanism. However, recent work on the genus *Neoconocephalus* relying on phylogenetic analysis to infer the sensory bias origin of a trait does not support the sensory bias hypothesis for the evolution of the leader preference. In this genus species exist with continuous and discontinuous calls, where the latter are the derived state (Snyder et al., 2009). With the exception of one species, males of *Neoconocephalus* with discontinuous calls synchronize their chirps with those of other males (Greenfield, 1990; Greenfield and Schul, 2008) (J. Schul, unpublished observations).

Contrary to the sensory bias hypothesis, some species with discontinuous calls exhibit a leader preference, while others do not (Greenfield and Schul, 2008; Bush and Schul, 2010). The authors consider the possibility that selection favouring accurate sound localization has reduced the amount of lateral inhibition, and as a consequence abolished the leader preference. This is consistent with our own finding that in the field cricket *Gryllus bimaculatus*, chirps presented in a leader–follower fashion do not result in a leader preference, and the AN1 neuron of the follower side is only weakly affected by the leader chirp (S. Hirtenlehner and H.R., unpublished observations). By contrast, in a pair of interneurons (AN1 neuron) in the locust *Schistocerca gregaria*, which receive strong contralateral inhibition (Römer et al., 1981), the presentation of *Mecopoda* leader–follower signals with a time delay of 70 ms resulted in the same asymmetrical bilateral response as shown for TN1 in Fig. 4B (M.E.S., unpublished). Future comparative studies on the strength of lateral inhibition will thus show whether this might explain the existence of female preferences in some species of a taxonomic group but not in others.

In the present study the interneuron TN1 showed strong asymmetrical responses to leader–follower stimulus conditions. In contrast to the local omega neuron in the prothoracic ganglion, TN1 could transmit the relevant information to the brain where decisions are likely to be made. The neuron has primarily been associated with the ultrasound-triggered negative phonotaxis in flight, based on its short response latencies, the match of its tuning curve with the tuning of avoidance behaviour in flight and the encoding of high pulse repetition rates of bat-like sound pulses (Faure and Hoy, 2000b; Faure and Hoy, 2000c; Libersat and Hoy, 1991; Schul and Schulze, 2001). However, the same neuron has also been suggested to be involved in positive phonotaxis in Tettigoniidae (Schul, 1997), and evidence from different members of Phaneropterinae suggests that the T-cell might function in mate detection for this group of katydids (ter Hofstede and Fullard, 2008; ter Hofstede et al., 2010). Furthermore, in the flightless Phaneropterine species *Leptophyes punctatissima*, the T-cell copies the extremely short clicks in the female reply (duration, 0.5 ms) with high reliability both in the laboratory and under field conditions (Kostarakos et al., 2007) (E. Ofner and H.R., unpublished). Similarly, in our study with *M. elongata*, TN1 copied the syllable pattern within the conspecific chirp with high accuracy and showed a similar response to a chorus signal lacking a distinct syllable structure (Fig. 1). The exact copy of the syllable period of the solo chirp together with the robustness of coding in natural background noise (see below) suggests that the pair of TN1 neurons might also play an important role in intraspecific communication in *M. elongata*. Thus, similar to the neuronal network for swimming in molluscs (Sakurai et al., 2011), homologous interneurons might have different roles in different species.

Lateral inhibition favours males signalling as leaders

Our experiments consistently revealed a strong and significant asymmetry in responses to identical chirps spatially separated by 180 deg, starting at time delays of about 20 ms, in favour of the leader signal (Fig. 3). This leader-biased response is transmitted to the brain of a receiver and may, therefore, represent the sensory basis of a behavioural preference for leader males in phonotaxis arena trials (Fertschai et al., 2007). The situation in *M. elongata* is thus different from a precedence effect studied in a cricket (Wyttenbach and Hoy, 1993), where a discharge difference in a pair of second-order auditory neurons (AN2) is completely absent; instead, the response in favour of the leader signal is generated in the brain.

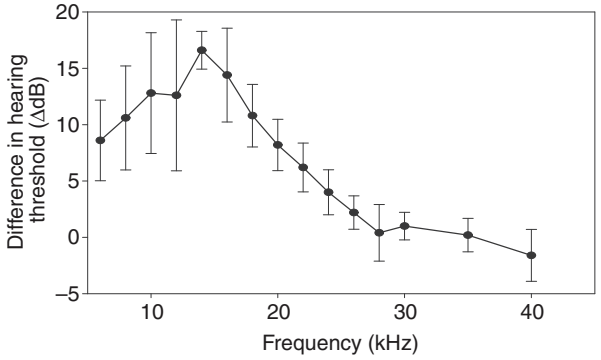

Fig. 8. Masked hearing thresholds of TN1 to pure-tone stimuli. Relative difference in TN1 threshold to pure tones presented in either the presence or the absence of native rainforest noise (mean ± s.d. of five preparations). Positive ΔdB values indicate a higher hearing threshold in the presence of noise.

The time delays that we used to examine leader–follower relationships are indeed those that occur in choruses of interacting males. Evidence comes on the one hand from playback experiments in which males were entrained to a repetitive conspecific signal broadcast with a steadily increasing signal rate. Under these conditions, males initiated their chirps 40–200 ms after signal onset (Hartbauer et al., 2005). On the other hand, in male duets, in which competitors differed by 190 ms in their solo chirp periods, the mean delay between leader and follower signals was 70 ms (Hartbauer et al., 2005). As our results show, such leader–follower situations evoked a significantly stronger response in the TN1 neuron on the leader side (Fig. 3A,B and Fig. 4A).

The stronger response to the leader signal is evident in the averaged values of response strength, but also in the trial-by-trail analysis, where we assumed that a female receiver cannot build an average response with signals produced at a relatively low rate of $0.5\,s^{-1}$, but rather uses the actual differences in response between the pair of neurons to make a decision to turn to one side or the other. In this analysis there is a win situation in about 90% of all responses, with very few wins for the follower, starting at 20 ms delay (Fig. 3B). Although these values decline for longer time delays approaching 250 ms (no overlap between the two solo signals), the advantage of the leader is still present. Importantly, the relatively small time delays between the two signals are strongly enhanced as a result of inhibitory interactions (see below), to create a ‘temporal contrast enhancement’ in the time of arrival of the two responses in the brain (Fig. 3C). This information would be available to create downstream commands for steering in addition to response strength differences.

The properties in the response asymmetries in leader–follower situations can be fully explained as a result of the synaptic input of the ipsilateral and contralateral neuron, known in the context of directional hearing [*Tettigonia viridissima* (Rheinlaender and Römer, 1980); *Neoconocephalus ensiger* (Faure and Hoy, 2000c)]. After elimination of the contralateral inhibitory influence, the representation of a follower signal is greatly enhanced (Fig. 5, upper traces). The complementary experiment of removing the inhibition from the follower side had no effect on the leader representation, as the follower exerted almost no inhibitory effect on the leader representation in the intact animal (Fig. 5, lower traces). Finally, contralateral inhibition and its interaction with excitation from the ipsilateral side would explain the trial-by-trial responses in the pair

of TN1 neurons (see recording examples in Fig. 3D). When the leader signal has a minimal temporal advantage, this auditory side is strongly excited and at the same time initiates a strong inhibition, which is forwarded to the contralateral TN1 neuron, long before the follower signal starts the excitatory action on this side. Thus, excitation due to the follower is elicited when the membrane potential is already strongly hyperpolarized, so that the first suprathreshold response is strongly delayed relative to the actual onset of the follower signal (see delayed responses in Fig. 3D at 70 ms delay). By contrast, without a delay, inhibitory and excitatory actions from the two sides interact at the same time, and in a bilateral symmetrical animal the outcome would strongly depend on random internal fluctuations, which explains why in this particular stimulus situation the strongest response often switches randomly between the two sides [for similar findings in the context of directional hearing, see studies by Rheinlaender, Römer and colleagues (Rheinlaender and Römer, 1980; Römer et al., 1981)]. Consistent with this view, a simultaneous presentation of conspecific signals from separated speakers resulted in a situation in which phonotactically responsive females turned to either speaker with equal probability (Fertschai et al., 2007). The origin of inhibition onto TN1 is presently unknown, but may be driven by the pair of omega cells in the prothoracic ganglion. In another katydid (*Ancistrura nigrovittata*) lateral inhibition of the AN1 neuron is mediated through omega cell activity (Stumpner and Molina, 2006).

Time–intensity trading of the leader advantage

The robustness of a preference for leader signals can be tested by increasing the SPL of the non-preferred signal up to a point of no preference, or the reversal of the preference. In this way, the behavioural preference for the leading signal was abolished in females of the frog *Hyla cinerea* when the SPL of the lagging signal was increased by 6 dB over that of the leading signal (Klump and Gerhardt, 1992). Similarly, a time–intensity trade off in synchronously calling males of this frog species (Dyson and Passmore, 1988; Höbel, 2010) revealed values of 9 dB to compensate for the advantage of the leading signal, and 4 dB in the katydid *Neoconocephalus spiza* (Snedden and Greenfield, 1998). In previous experiments with female *M. elongata*, a 6–10 dB increase of the follower signal (depending on the absolute SPL of the signals) was necessary to turn a leader preference into a preference for the follower signal, which corresponded well with bilateral discharge differences in the pair of omega neurons (Fertschai et al., 2007; Römer et al., 2002).

A similar interdependence of time delay and signal levels was found in the bilateral response of the pair of TN1 neurons, where follower signals fully compensated for the temporal advantage of the leader signal at 55 dB SPL with an increase in loudness of 8–10 dB (Fig. 6A). At 65 dB SPL, however, the advantage of the leader signal was much stronger (Fig. 6B), demonstrating that contralateral inhibition causes a very effective suppression of the neuronal response to the follower at higher signal levels. A 65 dB SPL would represent a sender–receiver distance of about 5 m, and the result of the time–intensity trading experiment would suggest that the ability to compensate for the follower's disadvantage in the signal representation in a receiver would depend on the density of the chorus.

TN1 response to conspecific signals under natural noisy conditions

Constraints imposed by physical and biological background noise are common to all communication systems. Either type of noise reduces the signal-to-noise ratio in receivers and limits the active space of a signaller (Klump, 1996; Brumm and Slabberkoorn, 2005). In the nocturnal rainforest, the natural habitat of *M. elongata*, frogs and crickets generate background noise in a frequency range between 3 and 9 kHz; additionally, various katydid and bat species generate transient high-frequency signals as well as ultrasound (Riede, 1997; Ellinger and Hödl, 2003; Balakrishnan, 2005; Lang et al., 2005; Hartbauer et al., 2010). This ambient background noise has the potential to mask advertisement signals of *M. elongata*, with their broad frequency spectrum between 5 and 90 kHz (Fig. 1A). It is therefore important to consider sensory coding under these more natural conditions. The TN1 response to conspecific signals presented at 65 dB SPL was only marginally affected by a simultaneous playback of low-pass filtered rainforest noise of the same intensity. This is probably due to a 30 dB roll-off in the TN1 tuning between 10 and 5 kHz (ter Hofstede et al., 2010). Such a high-pass filtering property of TN1 tuning has also been reported in various other bushcricket species (reviewed in Faure and Hoy, 2000b). Nevertheless, low-pass filtered rainforest noise reduced TN1 spiking activity significantly, either because of an adaptation at the receptor level caused by ongoing sensory stimulation by the sonic component (e.g. Gollisch and Herz, 2004) or as a result of additional inhibition by frequencies below 9 kHz. These mechanisms, together with an increase in hearing threshold in the frequency range below 25 kHz (Fig. 8) may contribute to a weak or absent response of TN1 to transient high-frequency signals in the background noise. As a consequence, conspecific signals are selectively encoded despite the presence of background noise. This response property is very different from the activity of omega cells in katydids, with their tonic responses copying amplitude modulations of almost all acoustic events in the background (Lang et al., 2005; Hartbauer et al., 2010). The selective encoding of conspecific signals by the TN1 neuron probably emphasizes its functional role in signal detection and species recognition in an acoustically complex habitat, which deserves further investigation.

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