biology letters
Biol. Lett. (2008) 4, 711–714
doi:10.1098/rsbl.2008.0367
Published online 19 August 2008
Neurobiology

Reliable coding of small, behaviourally relevant interaural intensity differences in a pair of interneurons of an insect

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Insects, as vertebrates and humans, use interaural intensity differences (IIDs) between the two ears for sound localization. They are remarkably sensitive for small IIDs of the order of 1–2 dB. Here, we investigated, using an independent ear stimulation paradigm, how such small IIDs are reliably encoded in the binaural discharge differences of a prominent pair of interneurons. Starting with an IID of 1 dB, these differences are large and significant, with the louder side being more strongly excited. In a comparison of simultaneous responses of left and right interneurons, more than 70 and 90 per cent correct responses occur at IIDs of 1 and 2.5 dB, respectively.

Keywords: sound localization; interaural intensity difference; dichotic stimulation; binaural cues

1. INTRODUCTION

Mate finding in insects involves both the recognition of sound signals and the localization of the signaller (Gerhardt & Huber 2002). As in vertebrates, these insects use binaural cues such as interaural intensity differences (IIDs) to accomplish the task of sound localization (Michelsen 1998; Pollack 2000; Robert & Göpfert 2002). Results obtained with independent (dichotic) ear stimulation in a bush cricket revealed a remarkable sensitivity for small IIDs of 1–2 dB (Rheinlaender *et al.* 2006). Such a high resolution was also found in a grasshopper (von Helversen & Rheinlaender 1988; Ronacher & Krahe 2000).

How are such small, behaviourally relevant IIDs reliably encoded in the discharge differences of afferents and interneurons of the auditory pathway? The high precision observed in behaviour is surprising given that (i) the neural circuitry for this task comprises only few receptors in each ear (between 20 and 100 in grasshoppers and bush crickets) and (ii) the high variability in the receptor responses (Stumpner & Ronacher 1991; Ronacher & Krahe 2000). At the level of interneurons as synaptic targets of auditory receptors, even fewer elements are available to code for these binaural differences. In their attempt to determine the neuronal correlates of such small IIDs, Ronacher & Krahe (2000) noted that an IID of 1.5 dB corresponds to a spike count difference of only 1 spike per 100 ms stimulus response in a bilateral pair of receptors, but that the error probabilities for a decision based on these differences are larger than those observed in behaviour. They therefore concluded that the insect has to integrate information from 6 to 13 receptors to arrive at the observed behavioural precision.

The present paper aims to follow this idea by studying the coding of small IIDs in a pair of identified first-order local interneurons in the auditory system of bush crickets. These omega neurons in crickets and bush crickets receive direct excitatory inputs from most of the auditory receptors in the ipsilateral foreleg (Römer *et al.* 1988), and inhibitory inputs from the contralateral side mediated by its homologous counterpart on the other side (Selverston *et al.* 1985; Molina & Stumpner 2005). Thus, a given IID produces a combined excitation and inhibition in each neuron, which is reflected in the spike count of both cells, rather similar to cells in the lateral superior olive and inferior colliculus of vertebrates (e.g. Park *et al.* 2004). Here, we take advantage of the possibility of recording the activity of both cells simultaneously and to determine spike count differences for each IID presented to the ears. Our results demonstrate that small, behaviourally relevant IIDs are available for these insects from simple spike count differences in pairs of auditory interneurons.

2. MATERIAL AND METHODS

Fourteen male and female *Mecopoda elongata* (Orthoptera; Tettigoniidae) were experimental subjects in neurophysiological experiments. Details of recording and analysing action potential activity of both omega neurons simultaneously have been described by Römer *et al.* (2002) for another bush cricket, enabling spike recordings from both cells with amplitudes sufficiently different for discrimination and separate analysis (figure 1*b*). Superposition of small and large action potentials occurred in less than 2 per cent, and therefore had a negligible effect on the results.

Dichotic stimulation of the ears allows to stimulate both ears independently and thus to apply precise IIDs. This was achieved using a method first described by Boyan (1983) for locusts. Acoustic stimuli were delivered independently to each auditory side via Motorola speakers (type PH10), to each of which a brass cone (length 35 cm) was attached. The end of the cone (final diameter 0.5 cm) was positioned close to the acoustic spiracles, which represent the main acoustic input to the ears. Calibration of each loudspeaker and cone assembly was achieved by placing a Bruel & Kjaer condenser microphone (type 4135) in front of the cone aperture. The crosstalk between the two ears was measured as described by Rheinlaender *et al.* (2006), and was at least 30 dB in each preparation. Thus, we could apply lateralized stimuli to either hearing organ without stimulating the opposite one up to 30 dB above threshold. Pure tone sound pulses (250 ms duration) at a carrier frequency of 20 kHz were used as stimuli, and broadcast at a rate of 1 s^{-1}.

The threshold for each side was determined in a monaural stimulation. These thresholds served as references for the dichotic stimulation, and all intensities are given in decibels above this threshold. IIDs up to 3 dB were applied to the system by either increasing or decreasing the sound pressure level (SPL) on only one side, or changing the SPL symmetrically, and in opposite directions, by a fixed amount on both sides. Each IID value was presented 15 times for later statistical analysis. Spike number and timing was evaluated in a time window of 300 ms following stimulus onset. All statistical tests were performed as a paired *t*-test, after control for normal distribution of the data in SigmaStat.

3. RESULTS

A typical example for the coding of small IIDs by the pair of omega neurons is shown in figure 1*a*, for a

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2008.0367 or via http://journals.royalsociety.org.

Received 4 July 2008
Accepted 31 July 2008

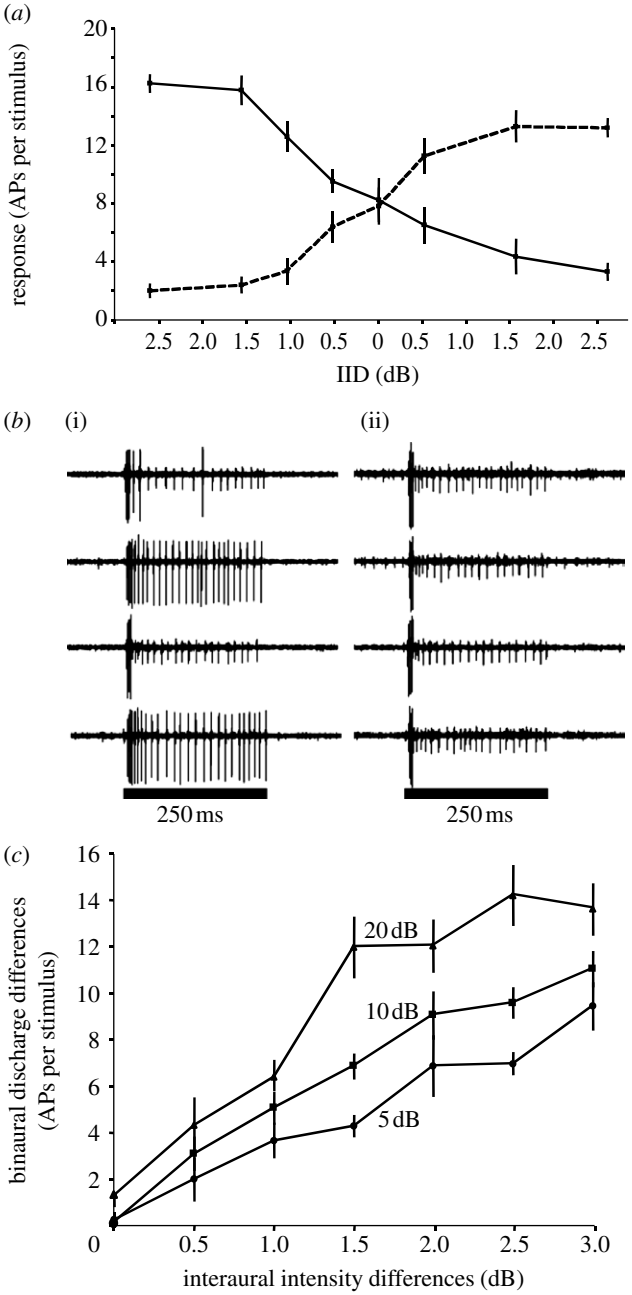

Figure 1. (*a*) Simultaneously recorded responses of both omega cells for small IIDs in one preparation (5 dB above threshold; means of 15 responses ± s.e.m.). (*b*) Examples of responses at (i) 0 dB and (ii) 3 dB IID, and 10 dB above threshold. Note the switching in response strength with symmetrical stimulation. (*c*) Mean discharge difference ± s.e.m. of all preparations ($n = 14$) with varying IIDs, separated for the three intensity levels tested. For further explanation see text.

SPL of 5 dB above threshold. When both ears were stimulated at the same level, the response of both cells was almost identical (approx. 8 spikes per stimulus). Increasing the SPL at the left ear by only 0.5 dB, while the SPL at the right ear was kept constant, resulted in a difference in the discharge of the omega pair, although the difference was not significant. A further increase of the IID to 1 dB and higher resulted in a significant difference in the responses. Although both cells exhibit the same response at an IID of 0 dB on average, they fluctuate strongly in their response (figure 1*b*(i)). However, as

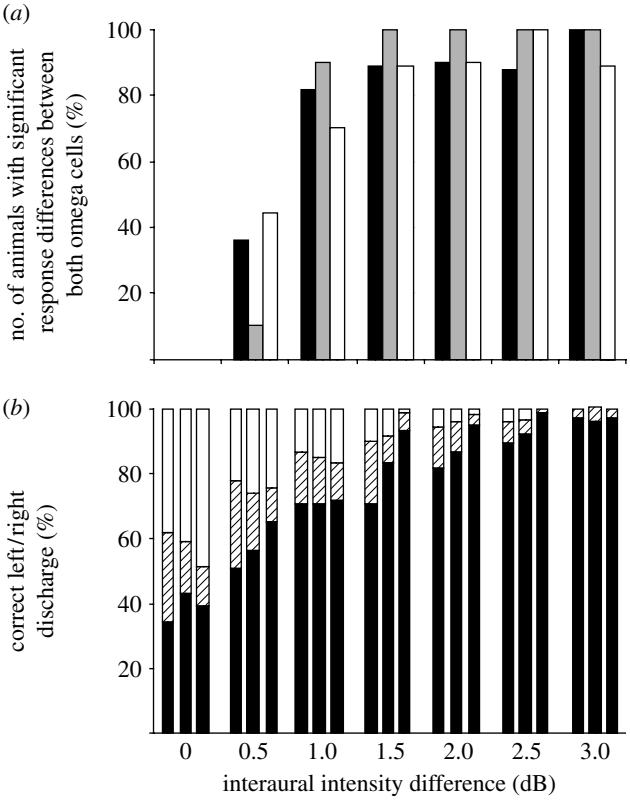

Figure 2. Analysis of binaural responses to small IIDs. (*a*) Percentage of preparations with significant stronger responses to the side leading in intensity (black bars, 5 dB above threshold; grey bars, 10 dB above threshold; white bars, 20 dB above threshold). (*b*) Classification of binaural responses into correct, equal and incorrect (black, hatched and white bars, respectively). Left, middle and right bars represent data for 5, 10 and 20 dB above threshold. For further explanation see text. Note that in the case of 0 dB IID, black and white bars just refer to left and right omega cells.

soon as these statistical fluctuations are superimposed onto an existing binaural difference of 3 dB, there is a reliable encoding of this difference, with the side leading in the SPL being more strongly excited (figure 1*b*(ii)). Figure 1*c* summarizes the data of all preparations as differences in spike counts, separated for the three intensity levels tested. These differences increase with increasing IID, and amount to 9.3 ± 3.1, 10.9 ± 2.4 and 13.5 ± 3.2 APs at IIDs of 3 dB and 5, 10 and 20 dB above threshold, respectively. Discharge differences at 20 dB above threshold are significantly higher compared with 5 dB above threshold, for IIDs of 1 dB and higher.

The results for the reliable encoding of small, behaviourally relevant IIDs are summarized for all preparations in figure 2 in two different ways. Figure 2*a* documents, for all intensity levels tested, that in 70–90% of the preparations a significant response difference was elicited in the pair of omega neurons with an IID of 1 dB, and with IIDs of 1.5–3 dB this was the case in 90–100% of preparations. Remarkably, in some preparations, significant differences already occurred at IIDs of 0.5 dB.

The analysis in figure 1*a* is based on averages over 15 stimulus presentations, and therefore implicitly assumes that receivers, when confronted with a given IID, somehow integrate neuronal activity over some

time before making a decision for a phonotactic turn. However, acoustic insects must often base their decision on single stimulus events, and therefore have to compare afferent binaural activity for each successive stimulus independently, before turning or moving towards the stronger stimulated side. We therefore analysed the data in a stimulus-by-stimulus fashion: only stimuli which elicited a response that was stronger by more than 10 per cent of the maximum discharge at a given SPL were designated as correct, if this happened for the more strongly stimulated side. The result of this analysis is shown in figure 2*b*, and indicates an increase in correct decisions based on these binaural discharge differences with increasing IIDs. Only minor differences exist between the three SPLs tested.

We also addressed the question of whether the temporal spike pattern, rather than the simple spike count, might enhance the discrimination of small IIDs, by calculating van Rossum distances of simultaneously recorded responses of both omega neurons (Machens *et al.* 2003). However, using a time constant of 5 ms for this analysis, significant differences occurred at IIDs of 1.5 dB and higher and 20 dB above threshold, indicating that spike count alone appears to be the best discriminating factor for small IIDs (for further information regarding the use of van Rossum metrics see the electronic supplementary material).

4. DISCUSSION

In insects, direction-sensitive interneurons with excitatory and inhibitory lateral inputs have been described, where both the timing and strength of inhibition determine their directionality (Pollack 2000). However, the number of neuronal elements that contribute to directional coding is small, since the ears comprise only a few receptors, and the number of interneurons providing ascending information to the brain may even be reduced (approx. 20 in grasshoppers or even one in the case of crickets; Schildberger & Hörner 1988; Stumpner & Ronacher 1991). Thus, the possibility for parallel processing by extracting directional information from responses of several interneurons is rather limited. It is therefore important that reliable information about IIDs is provided by the discharges of single pairs of interneurons, such as the omega neurons. These cells integrate the activity of almost all receptor cells, and the lateral contrast is enhanced by reciprocal inhibition.

Here, we have shown that small IIDs of the order of 1–1.5 dB, which create significant correct turns to the louder side in a bush cricket (Rheinlaender *et al.* 2006), produce reliable discharge differences in the pair of omega neurons. The ability to resolve such small IIDs is in good agreement with quantitative data on the accuracy of phonotaxis, where females experience only small stimulus angles (and thus IIDs of 1–2 dB; Rheinlaender *et al.* 2006). Discharge differences increase with intensity, and with an IID of 1.5 and 20 dB above threshold the error probability based on these spike discharges is approximately 5 per cent. Compared with the 'ideal observer' performance of a single pair of receptors for the same IID, stimulus duration (250 ms) and intensity level (15–20 dB above threshold), the discharge differences in the omega pair are much higher (approx. 13 spikes compared with 2.6 spikes per stimulus) and the error probability lower (5% compared with 17.5%; Ronacher & Krahe 2000). They concluded from their results on grasshopper receptors that substantial integration must take place in interneurons to explain the observed behavioural decision. Our results are consistent with this conclusion, since discharge differences increase (figure 1*c*) when more receptor cells are activated and their activity is integrated by the omega neuron.

Apart from receptor cell integration, the other mechanism for such an enhanced coding of IIDs is most likely the contralateral inhibition imposed by the homologous counterpart on the other side. Lateral inhibition has long been suggested as a common mechanism for processing small IIDs at the central nervous level, since it enhances subtle peripheral differences into large discharge differences of bilaterally homologous neurons. The reciprocal inhibition between the pair of omega neurons represents a special case of lateral inhibition in auditory neurons of Orthoptera, since even small statistical fluctuations in the excitatory level of one cell will be translated into a stronger inhibition of its counterpart (most evident with symmetrical stimulation at IIDs of 0 dB; figure 1*b*). Thus, both integration of excitatory postsynaptic potentials due to responses of several receptors and reciprocal inhibition result in reliable coding small IIDs in this pair of interneurons. In this way, behaviourally relevant IIDs (von Helversen & Rheinlaender 1988; Ronacher & Krahe 2000; Rheinlaender *et al.* 2006) are available for these insects from simple spike counts.

Funding was provided by the Austrian Science foundation, grant P17540-B06 to H.R.

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