Response patterns of two auditory interneurons in a freely moving grasshopper (Chorthippus biguttulus L.)

I. Response properties in the intact animal

Harald Wolf*
Institut für Zoologie II, Universität Erlangen, Staudtstrasse 5, D-8520 Erlangen, Federal Republic of Germany

Accepted February 17, 1986

Summary. Summated nerve potentials were recorded from the neck connectives in intact, freely moving grasshoppers of the species Chorthippus biguttulus by means of chronically implanted hook electrodes. The action potentials of two auditory interneurons, known as the G₁- and the B₁-neuron, respectively (Kalmring 1975 a, b), were distinguishable (Fig. 1) in the recordings and the neurons were identified by their morphology (Fig. 2).

The G-neuron exhibits a very rapid and another, much slower, response decrement; the times required for recovery from both these effects show the opposite time courses (Fig. 3). The response versus intensity curve of the G-neuron has the shape of a saturating characteristic for noise stimuli and high frequencies whereas at low frequencies inhibitory effects can be observed for high intensities. The B-neuron has a bell-shaped intensity characteristic at all frequencies with position and width of the bell being frequency-dependent (Fig. 5). The directional characteristic of the G-neuron is nearly circular (for noise stimuli); the B-neuron responds preferentially to sound from the ipsilateral side (Fig. 6). With increasing temperature the threshold, latency, and spike interval of the G-neuron strongly decrease, while the number of spikes per stimulus increases (Fig. 7).

In general, the response properties of both auditory interneurons as determined in almost intact Chorthippus biguttulus, largely resemble those previously reported for Locusta migratoria in extensively dissected preparations. However, a few, probably interspecific, differences were observed.

Introduction

Within the last two decades, the auditory pathway of grasshoppers has been the subject of detailed investigations (for reviews see e.g. Elsner and Popov 1978; Boyan 1984). Most of these studies have been performed in immobilized and dissected animals and usually assumed that the neural activity in these preparations would not differ much from that in the intact animal. For peripheral nerve fibres, such as the tympanal receptors which lack any efferent control, this hypothesis is most probably correct. In the case of central nervous interneurons, however, it seems worthwhile to make a detailed comparison of neural response properties in the intact and the dissected animal.

It is not known whether a motivation-dependent processing of auditory information takes place at the level of thoracic auditory interneurons (for instance, motivational gating in the case of auditory innate releasing mechanisms) or whether extensive removal of proprioceptive input in the course of preparation might affect the auditory pathway. It cannot be judged, therefore, to what extent the activity of a certain interneuron in the dissected animal will actually reflect physiological conditions. For instance, Heiter (1983) reports that a large visual interneuron, the DCMD, is inhibited before and during defensive kicking. This behavior-related control of responsiveness is abolished, however, by extensive dorsal dissection, even if the central nervous system itself remains completely uninjured.

It was the goal of the present study to determine the response characteristics of auditory interneurons in freely moving and almost intact grass-
hoppers and, moreover, in a species with pronounced acoustic behavior (D. and O. von Helversen 1983). Motivational influences on the auditory pathway are more likely to occur in these animals than they are in Locusta migratoria, a species which lacks comparable behavior but has been much studied previously.

Methods

Recordings were made from the neck connectives of Chorthippus biguttulus, the animals being captured around Erlangen (FRG) and kept in laboratory culture until at least one week after the final moulting. The method was partly adopted from Kämper and Dannbach (1981). Hook electrodes (steel wire with lacquer coating, 30 μm in diameter, insulation removed on the inner surface of the hooks, inside diameter of the hooks ca. 80 μm) were attached to the neck connectives while the animal was under carbon dioxide anaesthesia. The connectives were easily accessible by cutting a slit into the ventral membranous cuticle of the throat. The wire was strung up to the pronotum, fixed by a mixture of wax and resin (1:2) and suspended from a plug some 20 cm above the floor. This allowed the grasshopper freedom of movement on a large tussock of grass.

The electrodes implanted in the neck recorded summed nerve potentials in the microvolt-range; conventional amplification and recording techniques were applied. The action potentials of single large interneurons could be identified by their conspicuous amplitudes. To enable computer-assisted evaluation, the amplitude and time of occurrence of all spikes were digitized and stored on magnetic tape (Zarnack and Möhl 1977). To reduce the amount of data, amplitudes below a certain threshold (broken line in Fig. 1) were discarded before digitization, as was one (usually the negative) amplitude value of the biphasic spikes. In an amplitude histogram of all these spikes (Fig. 1) an amplitude window could be defined that corresponded to the activity of one particular neuron (e.g. between 0.8 and 1.0 for the G-neuron in Fig. 1) – a prerequisite for further evaluation. In the case of the B-neuron – which could only be recorded with small spike amplitudes – spikes from the ‘background activity’ not related to the stimulus were sometimes interspersed in the amplitude window set for evaluation, hence mimicking spontaneous activity of this fibre.

During the experiments the animal sat in a Faraday cage lined with sound absorbing material (cones of plastic foam), 50 cm in front of a loudspeaker (high pitch piece with ±3 dB linear frequency response between 3.5 and 40 kHz, for tones below 5 kHz dynamic loudspeaker with linear frequency response between 1 and 6 kHz). Echoes from the walls of the experimental chamber were at least 20 dB fainter than the stimulus itself. During acoustic stimulation the animal sat on a revolving piece of wood (50 x 4 x 1 mm) arranged ca. 3 cm above a ground of rock wool. Thus, to determine a directional characteristic, the animal was turned in a stationary sound field and not vice versa. Care was taken that the animal assumed its normal resting position, hindlegs cocked, during the measurements. Adjustment of the animal in the sound field was possible with an accuracy of ±3° – the accuracy being limited mainly by adjusting the animal parallel to the wooden rod which itself was mounted on a goniometer.

To record response versus intensity curves (called intensity characteristics, e.g. for threshold directional or frequency characteristics) 50 to 150 series of sound pulses (10-ms pulses, intervals 100 ms) of rising intensity (steps of 3 dB, between 40 and 88 dB) were presented. With this stimulus pattern habituation is almost negligible (except for the rapid response decrement in the G-neuron, see Fig. 3).

As a threshold criterion in intensity characteristics a 50% criterion was chosen (sound pressure level at which the stimulus was answered with a 50% probability, see Rheinlaender and Römer 1980). The term ‘threshold’ in itself shall mean the intensity value defined by the intersecting point of the prolongation of the ascending branch of the intensity characteristic with either the abscissa or some apparent ‘spontaneous activity’ level (see above). In the Figures standard deviations are always indicated. The terms ipsi- and contralateral always refer to the position of the hook electrode at the neck connective.

The temperature during the experiments, unless stated otherwise, was between 23° and 28°C.

Usually, the G- and B-neuron were identified by their unique physiological characteristics (see Results). These identifications were confirmed by determining the morphologies of both neurons (Fig. 2). During recording from the cervical connectives with the hook electrodes, a micropipette was used to probe the thoracic ganglia until a cell was found whose discharge matched that of the neuron of interest in the hook electrode recording. The cell was then injected with Lucifer Yellow (Wast et al. 1978) and identified by microscopic examination. In this way the auditory neurons appearing regularly in my recordings have been identified as the G1- and the B2-neuron, respectively, as described by Kalmar (e.g. 1975a, b). These identifications were performed in one animal only. In this paper I will retain the old, but generally familiar, terms G1- and B2- or simply G- and B-neuron. The G-neuron has also been called VS2 (Silver et al. 1980), TH2-TC1 (Hedwig 1985) and TN3 (Marquart 1985). The B-neuron has been termed AN1 (Römer and Marquart 1984) and TH3-AC1 (Hedwig 1985).
Results

General physiological characterization

The G-neuron of *Chorthippus biguttulus* responds not only to auditory stimuli but also to vibration applied to the fore and middle legs, as reported in *Locusta migratoria* (Cokl et al. 1977). The latency of this vibrational response ranges from 5 to 7 ms which is noticeably shorter than the 10 to 15 ms in the case of auditory stimuli (both classes of stimuli delivered at saturating intensities). The G-neuron shows a pronounced phasic response to auditory and vibratory stimuli. For long stimuli it is followed by a weak 'tonic' discharge consisting of single spikes with large and irregular intervals (2–30 ms).

For repetitive stimulation a clear decrement of the phasic response occurs (Fig. 3). The completely unhabituated neuron responds to a single short noise pulse (e.g. 70 dB, 2–10 ms) with an average of about 5 spikes. However, a second pulse within the next 100 ms (and at lower intensities may be a third pulse) only evokes one or two action potentials. The time required for recovery from this rapid habituation is in the range of several seconds.

A further decrement below the level of 1 to 2 spikes per stimulus occurs only at much higher stimulus rates and durations. The time course of this decrement is slower and recovery is more rapid than for the effect described before. It is not only the response level but the threshold as well that is affected by this habituation (Fig. 3B).

The response versus intensity curve of the G-neuron has the shape of a typical saturating characteristic (see Fig. 3B) – at least for high pitch
and noise stimuli (see Fig. 5). The threshold for noise stimuli is between 50 and 55 dB (in the partly habituated neuron, see Methods); the dynamic range extends up to 65 to 70 dB. The general response features of the G-neuron of *C. biguttulus* closely resemble those reported from *L. migratoria* (Kalmring 1975a, b), except for an apparently more accentuated phasic response in *C. biguttulus*, especially at low intensities (Eichendorff and Kalmring 1980).

The B-neuron is excited only by acoustic stimuli whereas vibratory input seems to have inhibitory effects (Cokl et al. 1977). Its response comprises phasic and tonic properties and is strongly dependent on stimulus intensity (Fig. 4). The latency of the B-neuron response is usually some 2 ms longer and the threshold for noise pulses about 6 dB lower than stated for the G-neuron. The response versus intensity curve is bell-shaped, that is, the neuron is inhibited by high sound intensities (above ca. 60–70 dB, see Fig. 4 and also in Wolf 1986, Fig. 2). Pronounced response decrements, as reported for the G-neuron, were not observed. The response properties of the B-neuron are quite similar to those found in *L. migratoria* (Kalmring 1975a, b), except that the threshold seems to be much higher in *Locusta* (Eichendorff and Kalmring 1980).

In both neurons, no differences in the response characteristics could be observed between the sexes.

**Frequency characteristics**

At frequencies above 14 kHz the G-neuron exhibits a typical saturating intensity characteristic like that already known for noise stimulation. Low frequencies (especially 4 to 6 kHz) delivered at high intensities cause pronounced inhibitory effects in the G-neuron (Fig. 5 upper diagram). By simultaneous stimulation with low and high frequency tones it could be demonstrated that the inhibition provided by a loud low pitch stimulus (e.g. 5 kHz and above ca. 60 dB) can effectively suppress the response to an even louder, simultaneously presented high frequency stimulus (e.g. 15 kHz, 60 dB, see Wolf 1984). This confirms the view of Kalmring (1975a) that high threshold low

---

**Fig. 3A–D. Habituation of the G-neuron. A Rapid habituation. When stimulating with a series of noise pulses (10 ms, 70 dB, intervals 30 ms, envelope in lower trace) after a long period of silence (ca. 30 s), the first stimulus is answered by several (in this example 4) spikes; this response level rapidly decreases to one to two spikes per stimulus. B Change in threshold. The animal was stimulated with series of noise pulses with rising intensity (see Methods, 64 series repetitions). These stimulus series were repeated in intervals of 16 s (open circles) or 1 s (filled circles). For the long stimulus-series interval – in the almost not habituated neuron (habituation starts at 58 dB or the 6th stimulus) – the threshold is about 9 dB lower than in the habituated cell. C Dependency of stimulus duration, intensity, and interval. The animal was stimulated with series of 14 noise pulses (intervals of series 2 s); within a series all parameters were kept constant but varied for subsequent series. Each combination of intensity, pulse duration, and interval was presented 16 times. The response to the first pulse depends on stimulus intensity only; the subsequent habituation is more pronounced with long pulses and short intervals. D Time course of disinhibition. Test stimuli (30-ms noise pulses, 70 dB) were presented in variable intervals after a strongly habituating stimulus (six 80-ms noise pulses, 70 dB, 10-ms intervals). Each point represents the average response to between 25 and 65 test stimulus presentations**
frequency receptors form inhibitory connections with the G-neuron.

The B-neuron exhibits its typical bell-shaped characteristic at all frequencies; the actual position and width of the bell are frequency-dependent, however (Fig. 5, lower diagram). Below 16 kHz the neuron responds to an intensity range of about 30 dB; at higher frequencies the shape of the bell becomes flatter and is only some 10 dB broad.

The G-neuron of *L. migratoria* responds preferentially within a ‘frequency-intensity-specific signal band’ which runs diagonally through the frequency-intensity field, originating at 5 kHz and 50 dB and terminating at 23 kHz between 87 and 97 dB, and which is consistently 10 to 15 dB broad (Kalmar and Rheinlaender 1974). This is distinctly different from the situation in *C. biguttulus*, in which the response area of the G-neuron is much larger, more homogeneously structured and is subdivided by inhibitory areas between 2 and 8 kHz only.

For the B-neuron of *L. migratoria*, Kalmar (1975a) reports a bell-shaped intensity characteristic at all frequencies as observed in *C. biguttulus*. The intensity range covered by the bell is slightly different, however (response between 40 and 60 dB at all frequencies, Kalmar and Rheinlaender 1974; or slow rise up to 80 dB above 15 kHz, Kalmar 1975a).

**Directional sensitivity**

The G-neuron exhibits a nearly circular directional characteristic in the transversal as well as in the
Fig. 6A–C. Directional characteristics of G- and B-neuron. Higher sensitivities are plotted at longer distances from the centre. 90° means ipsilateral with respect to the ascending axon (hook electrode position at neck connectives, see sketches of animals in the centres and Methods). A G-neuron, transversal plane. B G-neuron, frontal plane (during the experiments the animal sat on a wooden rod shielding sound from ventral, marked by black dots). C B-neuron, transversal plane

frontal plane (Fig. 6A and B). The sensitivity for ipsilateral stimulation is on the average 3 dB higher and for caudal stimulation some 3 dB lower than for the perfect circular shape. The thresholds indicated for stimulation from the ventral side range (150°–210° in the frontal plane, filled circles) have no significance since during the experiments the grasshopper sat on a small wooden rod (diameter slightly smaller than the animal’s body, ca. 3 mm) shielding sound from the ventral side.

The B-neuron exhibits a directional characteristic which is distinctly ipsilaterally oriented. Noise stimuli from the ipsilateral side are answered with a 10 to 12 dB higher sensitivity than stimuli from the contralateral side (Fig. 6C).

The nearly circular directional characteristic of the G-neuron indicates that this fibre must receive excitatory input from the ipsilateral as well as from the contralateral tympanal organ (Kalmring 1975a).

The ipsilateral orientation of the B-neuron’s characteristic suggests that this neuron receives excitation predominantly from the ipsilateral tympanal organ. The fact that the difference in sensitivity between ipsi- and contralateral stimulation is even larger than in the tympanal characteristic (see 2nd paper in this series Fig. 5; ca. 11 instead of 8 dB difference) further indicates the presence of an inhibitory influence from the contralateral tympanal organ. Römer and Rheinlaender (1983) have demonstrated that the B-neuron in L. migratoria receives excitatory as well as inhibitory input from both tympana. In fact, the B-neuron of C. biguttulus can be excited by exclusive contralateral stimulation. It still responds to sound even after the ipsilateral tympanal nerve has been cut (see Wolf 1984).

Temperature

All response parameters of the G-neuron examined – number of spikes per stimulus, latency, spike interval, and 50%-threshold – show strong changes with change in temperature. The number of spikes per stimulus rises from about 1.0 to 2.3 between 15 and 28 °C and remains constant at higher temperatures (Fig. 7A). The latency decreases from 21 to 6 ms between 15 and 40 °C (Fig. 7B) and the spike interval from 4 to less than 1 ms between 17.5 and 41 °C (Fig. 7D). The standard deviations of both parameters show parallel decrements. At 15 °C no spike interval could be determined because at this temperature stimuli elicited single spikes only (see Fig. 7A). The 50%-threshold changes by 25 dB between 15 and 40 °C, that is, by about 1 dB/°C (Fig. 7C).

Abrams and Pearson (1982) report that both current and voltage threshold of the intracellularly-impaled G-neuron increase slightly with temperature. Hence they conclude that the rise in the neuron’s response to acoustic stimuli with temperature is due to presynaptic elements, interneurons or auditory receptors. For the latter these authors and Ronacher and Römer (1985) report a marked increase in the response to sound stimuli with temperature.

Assuming that the marked temperature-related change of all response parameters of the G-neuron applies in a similar way to other auditory interneurons, as the results from tympanal receptors suggest, this change should be considered when electrophysiological data are compared with behavioral experiments. Electrophysiological examinations are usually performed at room temperature, behavioral studies, in contrast, mostly at 30
Fig. 7A–D. Response of the G-neuron as dependent on temperature. The diagrams (A) to (D) all present data obtained from one individual (different animals being quite similar, again). Latency, spike interval, and response in spikes per stimulus all were determined at saturating intensities (70 to 88 dB, see Fig. 3 B, inserted Fig. in (B) and Methods)

to 35 °C, the preferential temperature of many insects.

Discussion

The response characteristics of G- and B-neuron as determined in C. biguttulus largely resemble those previously reported for L. migratoria. This was to be expected considering the close morphological similarities of the neurons in both species (see Fig. 2 and Marquart 1985) but is remarkable considering that most of the previous studies have been performed on extensively dissected preparations whereas the results presented in this paper were obtained from almost intact animals. Obviously, the dissection alters the response properties of these neurons only marginally, if at all.

This clear lack of, for example, motivational influences on the response characteristics of G- and B-neuron could have two possible reasons: (i) at the level of these somewhat peripheral auditory interneurons such motivational gating has not yet taken place (but would occur at more central levels), or (ii) the G- and B-neurons are involved in neural networks controlling escape behavior (Wolf 1986) and are not subject to motivational influences at all because escape reflexes have the highest priority for survival.

The minor differences that were observed in the response patterns of G- and B-neuron in C. biguttulus compared to those of L. migratoria are probably due to interspecific variations, although effects of extensive dissection on the receiver characteristics of the tympanal organ, with ramifications on the frequency response and directionality of single neurons, cannot be excluded completely (Miller 1977). According to the much smaller body (and tympana) size of C. biguttulus compared to L. migratoria (ca. 20 mm versus 50 mm length) somewhat higher best frequencies, decreased directional sensitivity and higher thresholds of auditory neurons could be expected (Michelsen and Nocke 1974). The results presented above do not always agree with these expectations, however. The most prominent difference, concerning the structure of the G-neuron’s frequency-intensity field (Fig. 5),
suggests largely different projections of tympanal receptor fibres onto the G-neuron in the two species C. biguttulus and L. migratoria.

Acknowledgements. I am very grateful to D. and O. von Helversen who supported my work generously on all its stages and to B. Ronacher and K.-G. Heller for many stimulating discussions. I thank my wife for her valuable assistance, especially with finishing the Figures, K. Worley for her qualified support in translation and K.G. Pearson and I.C. Gynther for critical reading of the manuscript. The identifications of G- and B-neuron were performed together with V. Marquart (Bochum). Part of this work was sponsored by a scholarship from the Studienstiftung des Deutschen Volkes.

References

Kalmring K (1975a) The afferent auditory pathway in the ventral cord of the migratory locust. I. Synaptic connectivity and information processing among the auditory neurons of the ventral cord. J Comp Physiol 104:103–141