

## REPRESENTATION OF BEHAVIORALLY RELEVANT SOUND FREQUENCIES BY AUDITORY RECEPTORS IN THE CRICKET *TELEOGRYLLUS OCEANICUS*

GERALD S. POLLACK\* AND ZEN FAULKES

Department of Biology, McGill University, 1205 Avenue Dr Penfield, Montreal, Quebec, Canada H3A 1B1

\*e-mail: gpollack@bio1.lan.mcgill.ca

Accepted 21 October 1997; published on WWW 9 December 1997

### Summary

*Teleogryllus oceanicus* is particularly sensitive to two ranges of sound frequency, one corresponding to intraspecific acoustical signals (4–5 kHz) and the other to the echolocation cries of bats (25–50 kHz). We recorded summed responses of the auditory nerve to stimuli in these two ranges. Nerve responses consist of trains of compound action potentials (CAPs), each produced by the summed activity of a number of receptor neurons. The amplitude of the CAP is up to four times larger for stimuli at 4.5 kHz than for stimuli at 30 kHz, suggesting either that the extracellular spikes produced by receptors that respond to 4.5 kHz are larger than those that respond to 30 kHz, or

that receptors fire more synchronously in response to stimulation at 4.5 kHz, or that more receptors respond to stimulation at 4.5 kHz. Neither unit spike amplitude nor conduction velocity (which is expected to vary with spike amplitude) differs for the two frequencies, and the responses to 4.5 kHz are not produced by more tightly synchronized receptor populations, as judged by CAP breadth. We conclude that more receptors respond to 4.5 kHz than to 30 kHz.

Key words: acoustic communication, ultrasound, sensory coding, compound action potential, cricket, *Teleogryllus oceanicus*.

### Introduction

The ears of animals are often specialized to listen to sounds that are particularly relevant to their way of life. In the cricket species *Teleogryllus oceanicus*, two distinct frequency ranges are important. Intraspecific acoustic signals, which are used to attract mates, to induce copulation and in agonistic encounters, are dominated by frequencies in the range 4–5 kHz (Balakrishnan and Pollack, 1996). Aerially feeding echolocating bats produce signals with much higher frequencies, generally above 20 kHz (Simmons *et al.* 1979), to hunt insects. Crickets, like many other insects, can hear these high-frequency sounds, which trigger evasive behavior (Hoy, 1991; Hoy and Robert, 1996). Both behavioral and neurophysiological studies demonstrate that crickets are particularly sensitive to sounds in these two frequency ranges. For example, the behavioral audiogram describing the frequency-sensitivity of flight phonotaxis of *T. oceanicus* shows two low-threshold regions, one near 5 kHz and a second near 30 kHz (Moiseff *et al.* 1978). Similarly, most auditory interneurons in this species show increased sensitivity to one or both of these frequency ranges (Moiseff and Hoy, 1983; Atkins and Pollack, 1986, 1987*a,b*).

On the basis of recordings from interneurons, Pollack (1994) proposed that sounds in the lower frequency range, 4–5 kHz, are detected by many auditory receptor neurons, while ultrasounds are detected by only a few receptor neurons. In the present work we examine this hypothesis by recording from the auditory nerve.

### Materials and methods

#### Preparation

*Teleogryllus oceanicus* were raised in a laboratory colony on a diet of Purina Cat Chow with *ad libitum* access to water. Virgin females, aged at least 9 days after the last molt, were mounted on a wax block ventral side uppermost. The forelegs were fixed with the tibia and tarsus flexed against the femur, similar to their position during flight. However, to provide access to the anterior surface of the femur for electrode implantation, the coxae were rotated so that the legs, which in flight are held against the lateral surfaces of the body, projected laterally nearly at right angles to the longitudinal axis of the cricket.

Auditory receptor neurons were recorded *en masse* with Teflon-insulated silver-wire electrodes (o.d. 114 µm). The axons of the receptor neurons travel from their origin in the prothoracic tibia to the central nervous system in branch TB of N5 (Eibl and Huber, 1979). In the femur, branch TB lies on the anterior-dorsal surface of the main leg trachea, which is visible through the intact cuticle. Small holes were made in the cuticle with a pin, using the trachea as a guide, and electrodes were inserted until their tips nearly touched the trachea. To measure conduction velocity, we recorded simultaneously at two sites along the length of the femur separated by approximately 2 mm. The indifferent electrode was placed proximally and ventrally in the femur.

Individual receptors were recorded in N5 at a point close to its entry into the prothoracic ganglion using glass

microelectrodes filled with 3 mol l<sup>-1</sup> KCl (resistance 20–100 M $\Omega$ ). The ganglion was supported on a steel plate and bathed in physiological saline (Strausfeld *et al.* 1983). Experiments were performed at room temperature (20–25 °C).

### Stimuli

Sound pulses were produced with National Instruments A/D–D/A boards (12-bit resolution; update rate  $\geq 200$  kHz) controlled by software written using LabWindows. Unless otherwise noted, pulses had rise and fall times of 0.2 ms and an overall duration of 20.4 ms. Signals were amplified (Amcron) and broadcast either through piezoelectric tweeters (Motorola) or through 4 inch magnetic loudspeakers (Intertan). Stimuli were presented ipsilateral to the recorded nerve. Carrier frequency was either 4.5 kHz or 30 kHz, and intensity was varied (using either a custom-built programmable attenuator from Mike Walsh Electronics or Tucker-Davis Technologies PA4) from 25 dB to 100 dB in steps of 5 dB. Each frequency–intensity combination was delivered 50 times at a repetition rate of 2 s<sup>-1</sup>. The stimuli were given in separate series of intensity steps, one ranging from 25 to 95 dB in steps of 10 dB, and the other ranging from 30 to 100 dB. In most experiments the curves relating response magnitude and stimulus intensity were smooth when the measurements from the two series were interleaved, indicating that responses and recordings were stable throughout the experiment. Two experiments failed to meet this criterion and were discarded.

### Data analysis

Recordings were digitized (12-bit resolution, sampling rate 10 kHz per channel) using National Instruments A/D–D/A boards and the program SWEEPS (Pollack, 1997), which was also used for data analysis. Statistical tests were performed with SYSTAT or STATISTICA software.

In the Results section, we compare compound action potentials (CAPs) recorded from the auditory nerve in response to 4.5 kHz and 30 kHz sound stimuli. To avoid confounding frequency-dependent and intensity-dependent effects, most comparisons are made at equivalent sound intensities relative to threshold. Clear CAPs, which depend on the synchronous activity of many receptors (see below), are often not apparent until intensity is well above the minimum necessary to elicit auditory responses in the nerve as determined by visual inspection of the recordings. We define threshold not on the basis of CAPs but on the integrated responses of the nerve. We integrated recordings (following full-wave rectification) over two 50 ms time windows, one representing the response to sound, beginning at stimulus onset, and the second, a control measurement, beginning 100 ms later. The response was defined as the difference between the two measurements. Threshold (T) was defined as 5 dB greater than the most intense stimulus for which the mean of all responses ( $N=27-50$ ) did not differ significantly from zero ( $P>0.05$ ,  $t$ -test). (Responses to some sound pulses were obscured by bursts of motor activity that masked the much smaller auditory potentials. These responses were excluded from the analysis, resulting in lower

sample sizes.) Threshold was 55.3 $\pm$ 5.8 dB SPL at 4.5 kHz and 69.3 $\pm$ 8.4 dB at 30 kHz (mean  $\pm$  s.d.,  $N=15$ ). These values are 10–15 dB higher than behavioral thresholds and than the thresholds of some auditory interneurons (see, for example, Moiseff *et al.* 1978; Moiseff and Hoy, 1983; Atkins and Pollack, 1986), probably because of the masking of responses to low-intensity stimuli by ongoing, non-sound-related spiking in the nerve.

## Results

### Compound action potentials represent synchronous activity of receptors

Recordings from the whole nerve exhibit periodic compound action potentials (CAPs) (Fig. 1A). Similar periodic waveforms have been recorded in a number of other insects (acridids, Adam, 1977a; tettigoniids, Fullard *et al.* 1989; Rössler *et al.* 1990; cicadas, Huber *et al.* 1980; blattids, Shaw, 1994; Coleoptera, Yager and Spangler, 1995). The origin of the waveform was carefully studied in the locust by Adam (1977b). He found that different receptors have similar minimum latencies and maximum firing rates, and that periodic CAPs reflect the nearly synchronous activity of those receptors that have attained these conditions. Fig. 1 shows that such receptor synchronization explains cricket CAPs. Fig. 1A shows the response of the whole nerve and Fig. 1B shows a simultaneous recording from a single receptor cell recorded at the point where the nerve enters the central nervous system; this trace has been shifted a few milliseconds to the left to align the first spike with the first peak in the CAP. As the response proceeds, the single-unit spike becomes increasingly out of register with the CAPs, suggesting that synchronization among receptors decays with successive spikes. Accordingly, CAPs become progressively smaller throughout the response.

We analyzed in detail only the first CAP of a response, where synchronization among receptors appears to be greatest (see below). Thus, our measurements capture responses of receptors whose first spikes arrive at the recording electrode with similar latency, no matter what their firing rates. Indeed,

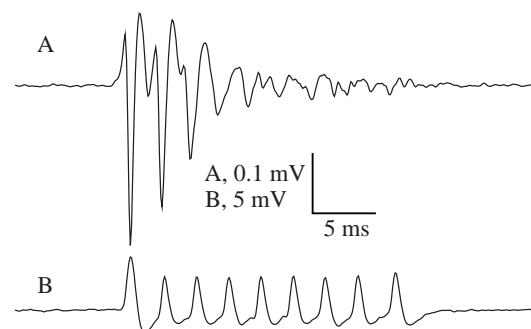


Fig. 1. Correspondence between single-unit spikes and compound action potentials (CAPs). (A) Average of 40 responses of the entire auditory nerve to a 4.5 kHz, 90 dB stimulus; (B) a single response from one receptor neuron from the same individual. The trace in B has been shifted to the left to align the first spike and the first CAP.

at lower stimulus intensities, often only a single clear CAP is evident in averaged recordings, indicating either that the contributing receptors produced only one spike or that their firing rates were sufficiently different to preclude synchronization except at the beginning of the response.

#### *CAPs are larger at 4.5 kHz than at 30 kHz*

Averaged recordings from the auditory nerve are shown in Fig. 2A,B. At low intensity, responses to 4.5 kHz and to 30 kHz are similar in amplitude. As intensity increases, however, the amplitudes of responses to 4.5 kHz increase markedly, while those of responses to 30 kHz increase only slightly. The results of several experiments are summarized in Fig. 2C. At a stimulus intensity 30 dB above threshold, CAPs evoked by 4.5 kHz stimuli are more than four times larger than those evoked by 30 kHz stimuli.

One hypothesis to account for these findings is that more receptor neurons respond to 4.5 kHz stimuli than to 30 kHz stimuli. CAPs increase with intensity as additional receptors are recruited but, because the pool of available receptors is, according to this hypothesis, larger for 4.5 kHz stimuli, the total number recruited will be greater, resulting in larger CAPs. However, CAP amplitudes could also differ because receptors that respond to 4.5 kHz produce larger unit spikes, which would sum to produce larger CAPs, or because they are

more tightly synchronized, resulting in more effective summation. We now examine each of these possibilities in turn.

#### *Amplitudes of unit spikes*

Fig. 3 illustrates single-unit recordings from two receptors, one that responded strongly to 4.5 kHz but only weakly to 30 kHz, and one that responded to 30 kHz but not to 4.5 kHz. In this paper, we classify receptors simply as either low- or high-frequency types, according to their strength of response (i.e. number of spikes elicited) to these two frequencies. A more detailed treatment of the physiological characteristics of receptors will be presented elsewhere (Imaizumi and Pollack, 1997, and in preparation).

To measure the amplitudes of single-unit spikes in the whole-nerve recording, we recorded simultaneously from single units and from the whole nerve. We visualized the spike in the whole-nerve recording of the neuron recorded as a single unit by searching for events in the whole-nerve recording that preceded the single-unit spikes with constant latency (Fig. 4A). Because the spikes of different receptors are synchronized at the beginning of responses, the events revealed using this method might include not only the single unit recorded with a microelectrode but also spikes from other, synchronously firing, receptors. We used two procedures to avoid this. When

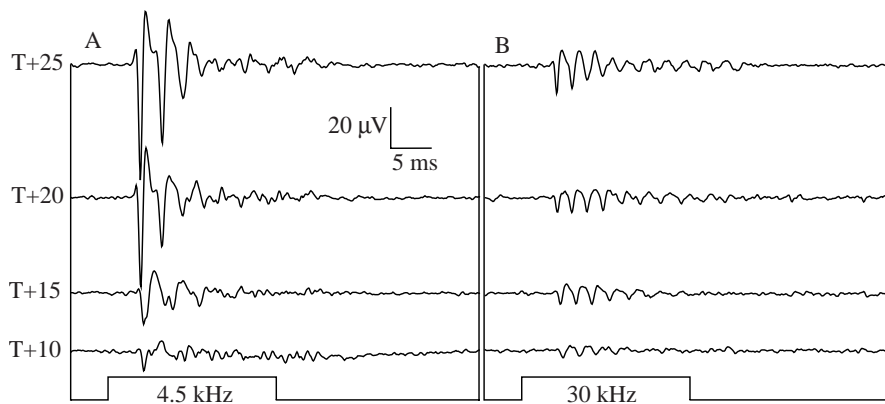
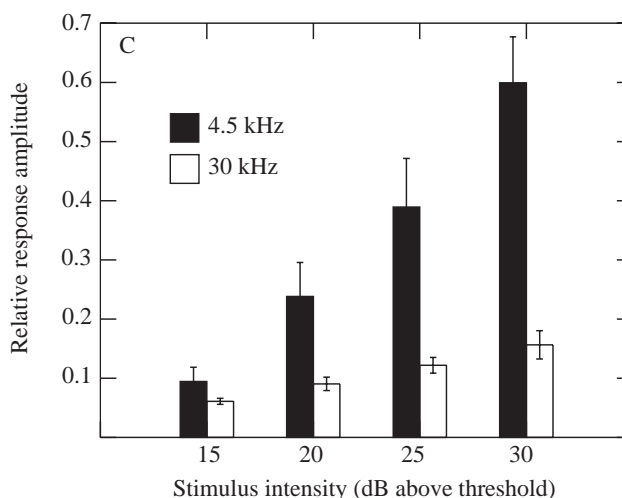


Fig. 2. Compound action potential (CAP) amplitude depends on stimulus intensity and on sound frequency. The traces show 31–47 averaged responses to stimuli at 4.5 kHz (A) and 30 kHz (B); the stimulus marker is indicated at the bottom. Stimulus intensities are indicated as dB above threshold (T), which in this preparation was 60 dB for 4.5 kHz and 70 dB for 30 kHz. (C) Mean ( $\pm$  S.E.M.) CAP amplitudes for 11 preparations. Amplitude was measured at the negative peak of the first CAP in each response (see inset, Fig. 8). In each preparation, amplitudes were normalized with respect to the first CAP evoked by 4.5 kHz, 100 dB. See Materials and methods for a definition of threshold. Response amplitude varied with stimulus intensity (ANOVA,  $F=10.0$ ,  $d.f.=3$ ,  $P<0.001$ ), and differed for 4.5 kHz and 30 kHz ( $F=29.8$ ,  $d.f.=1$ ,  $P<0.001$ ). In addition, the intensity-dependence of the response differed for the two frequencies (interaction between intensity and frequency,  $F=3$ ,  $d.f.=3$ ,  $P<0.001$ ). Sample sizes (for 15, 20, 25 and 30 dB respectively) at 4.5 kHz, 13, 14, 13 and 13; at 30 kHz, 11, 10, 9 and 8.



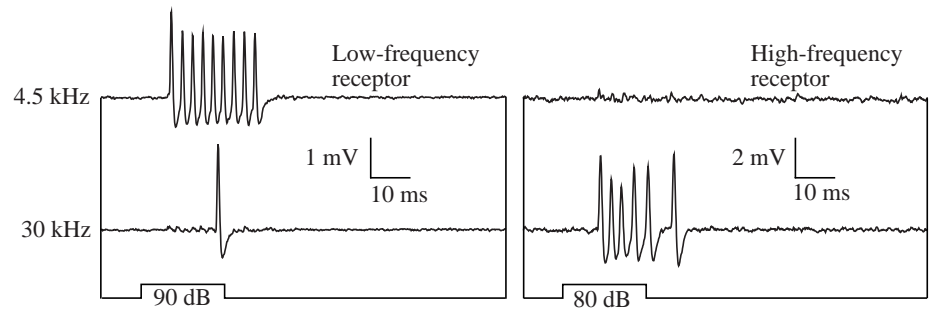


Fig. 3. Responses of typical low-frequency and high-frequency receptors.

possible, we used the spontaneous activity of receptors to visualize their spikes in the whole-nerve recording. For receptors that were not spontaneously active, we stimulated with 200 ms sound pulses. Periodic CAPs, indicative of synchronization among receptors, are evident only during the initial portion of the response (Fig. 4B). Accordingly, we use the last half of the response to visualize spikes in the whole-nerve recording.

We measured the amplitudes in the whole-nerve recording of spikes of both low- and high-frequency receptors in six preparations. To minimize inter-animal differences in the quality of the whole-nerve recording, we expressed unit spike amplitudes relative to the mean of all of the low-frequency units ( $N=1-4$ ) recorded in the same preparation. The mean normalized spike amplitude of high-frequency receptors,  $1.16 \pm 0.79$  (mean  $\pm$  s.d.) did not differ significantly from that of low-frequency receptors [ $1.0 \pm 0.40$ ; 11 low-frequency receptors and eight high-frequency receptors recorded in six preparations; analysis of variance with weighted means (Searle, 1987),  $F=0.5$ , d.f.=1,  $P=0.52$ ].

Axon diameter is an important determinant of spike amplitude in extracellular recordings (Pearson *et al.* 1970). Other things being equal, spike amplitude is expected to be proportional to the square of axon diameter (Rushton, 1951). Larger axons also conduct spikes more rapidly, with conduction velocity predicted to be proportional to the square root of diameter (Hodgkin, 1954; Pearson *et al.* 1970). Thus, conduction velocity is an indirect measure of spike amplitude. We measured conduction velocity by making whole-nerve recordings at two sites and measuring the conduction delay between them. The results are shown in Fig. 5. There is no difference in conduction velocities of CAPs evoked by 4.5 kHz and 30 kHz stimuli (Fig. 5A; ANOVA,  $F=0.058$ , d.f.=1,  $P=0.81$ ). There is a slight, but significant ( $F=4.18$ , d.f.=3,  $P<0.01$ ), increase in conduction velocity with increasing intensity, suggesting that receptors with larger-diameter axons may have higher thresholds. For the six preparations in which we could compare conduction velocities of low- and high-frequency receptors, we again found no difference (Fig. 5B, ANOVA with weighted means,  $F=0.124$ , d.f.=1,  $P=0.75$ ).

#### *Synchronization among receptors*

In this section, we examine the hypothesis that the amplitude difference between CAPs produced by 4.5 kHz and 30 kHz

stimuli is due solely to differences in the degree of spike synchronization among receptors. A direct method of studying synchronization would be to record simultaneously from several single receptors, but this is not technically feasible. Instead, we measured the widths of CAPs as an indicator of the degree to which receptors are synchronized. If the smaller amplitudes of CAPs evoked by 30 kHz stimuli were due to poor synchrony among the contributing receptors, then these CAPs would be broader than the larger CAPs elicited by 4.5 kHz. In

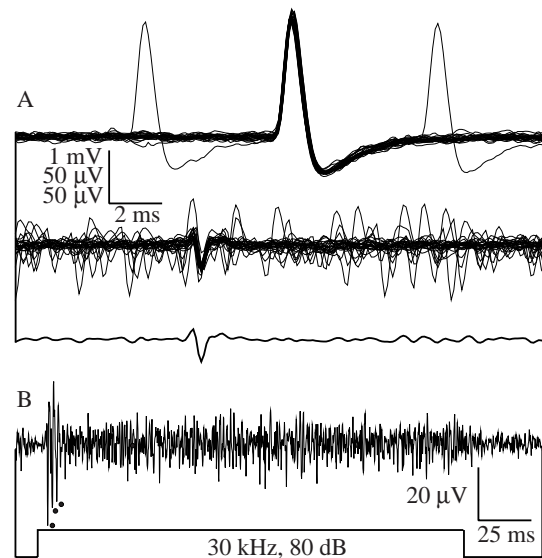


Fig. 4. Method for measuring single-unit spike amplitudes in the whole auditory nerve. (A) Twenty-six segments of a recording, showing a single-unit recording (top) and the associated whole-nerve recording (middle), are aligned according to the single-unit spikes and then superimposed. The bottom trace shows the average of the middle traces. The spike of the receptor recorded in the top trace can be resolved as a single unit in the whole-nerve recording because of the constant latency between its arrival at the nerve recording electrode and its arrival at the more proximal microelectrode. The recording segments were derived from several seconds of spontaneous activity. (B) For receptors that were not spontaneously active, spike-aligned sweeps were extracted from responses to long-duration stimuli. The trace shows the averaged whole-nerve response to 20 stimuli (the single-unit microelectrode recording is not shown). Clear compound action potentials (CAPs), indicating synchronous activity, occur only during the initial portion of the response (circles). Spike-aligned sweeps were selected from the second half of the response.

Fig. 5. Conduction velocities do not differ for responses to 4.5 kHz and 30 kHz. (A) Conduction velocities of compound action potentials (CAPs). The difference in arrival time of the first CAP of averaged responses at two recording sites was measured as shown in the inset. Within each preparation, conduction velocities were normalized with respect to the response to 4.5 kHz, 100 dB (which, for 12 preparations, was  $1.9 \pm 0.2 \text{ m s}^{-1}$ , mean  $\pm$  s.d.). Conduction velocities of CAPs evoked by 4.5 kHz and 30 kHz did not differ (ANOVA with weighted means,  $F=0.058$ , d.f.=1,  $P=0.81$ ). Conduction velocity did vary significantly with stimulus intensity ( $F=4.18$ , d.f.=3,  $P=0.009$ ), but there was no significant interaction between frequency and intensity ( $F=1.8$ , d.f.=3,  $P=0.153$ ). Sample sizes (for 15, 20, 25 and 30 dB respectively) at 4.5 kHz, 11, 12, 11 and 13; at 30 kHz, 11, 10, 8 and 8. (B) Conduction velocities of 19 single units, recorded in six preparations, normalized with respect to the mean conduction velocity of all of the low-frequency units recorded in a given preparation ( $N=1-4$  per preparation, 11 in total; conduction velocity  $2.1 \pm 0.4 \text{ m s}^{-1}$ , mean  $\pm$  s.d.). There is no significant difference in conduction velocity between low- (LF) and high-frequency (HF) receptors (ANOVA with weighted means,  $F=0.124$ , d.f.=1,  $P=0.75$ ).

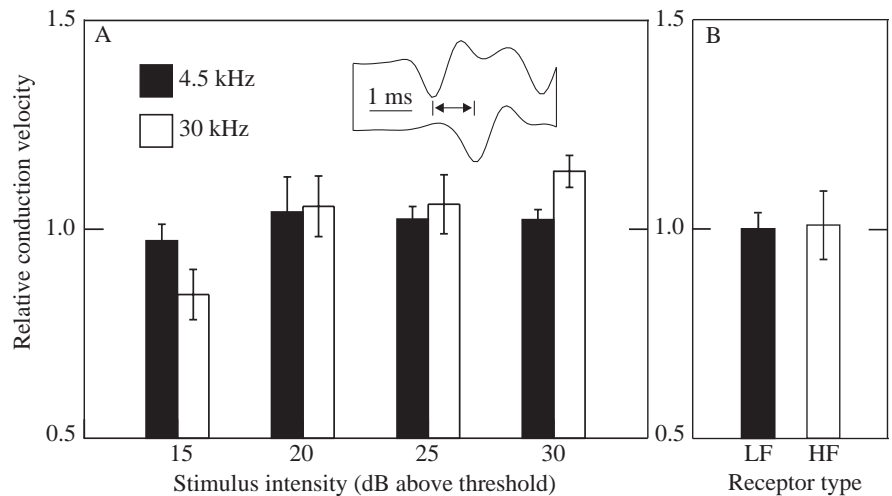


Fig. 6A, CAPs evoked by both low- and high-frequency stimuli are superimposed. The traces are the same as those in Fig. 2A,  $T+30$ , rescaled to allow superposition. The 30 kHz response is not broader than the response evoked by 4.5 kHz. In contrast, successive CAPs within a single response, where receptor synchronization presumably deteriorates with time (Fig. 1), do become broader as they decrease in amplitude (Fig. 6B), supporting the validity of this approach.

We compared the measured relationship between CAP amplitude and breadth with the relationship predicted by a model that produced artificial CAPs from a population of single-unit spikes that were identical in size and shape, but were distributed in time according to a Gaussian distribution. By adjusting the standard deviation of the Gaussian distribution (range 0–1 ms), we adjusted the degree of synchronization among the receptors. Each model CAP was produced by summing, at each point in time, the instantaneous voltages of each of 25 contributing single units (preliminary tests showed that the output of the model was similar with five units), and CAP amplitude and breadth were measured from the average of 50 model CAPs, each produced using the same standard deviation of spike timing.

The model was run separately for nine preparations, each time using a spike of an appropriate shape for that preparation. Rather than using actual single-unit action potentials (which were available only for a subset of the preparations and which differed slightly from receptor to receptor, even within a single preparation) to determine the unit shape, we used instead the first CAP in the response to a 4.5 kHz, 100 dB stimulus. Fig. 7 shows the spikes of four single units recorded in a single preparation, visualized in the whole-nerve recording with averaging (see Fig. 4), together with the CAP evoked by 4.5 kHz, 100 dB. The shape of the CAP is similar to the shapes of the single-unit spikes, suggesting that this intense stimulus

evokes highly synchronized activity among receptors. The half-widths of CAPs (duration at 50% of maximum amplitude; see inset to Fig. 8) in response to 4.5 kHz, 100 dB did not differ significantly from the half-widths of single-unit spikes recorded in the same preparations (15 single units recorded in five preparations; ANOVA with weighted means,  $F=1.88$ , d.f.=1,  $P=0.2$ ). In some preparations, the second positive phase of the CAP failed to return to the baseline before the arrival of the initial positive phase of the second CAP. In these cases, the

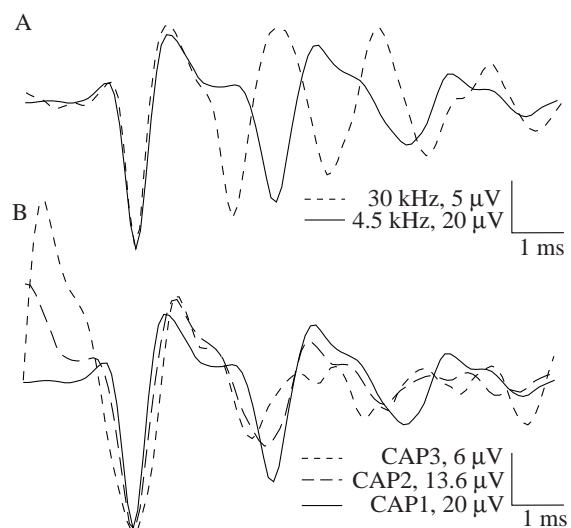


Fig. 6. Breadths of compound action potentials (CAPs). (A) The first CAPs elicited by 4.5 kHz (solid line) and 30 kHz (dashed line) stimuli, each 30 dB above threshold, do not differ in breadth despite their difference in amplitude (note scale). (B) Successive CAPs within a single response (to 4.5 kHz, 30 dB above threshold) are both smaller and broader. Solid line, first CAP; long-dashed line, second CAP; short-dashed line, third CAP.

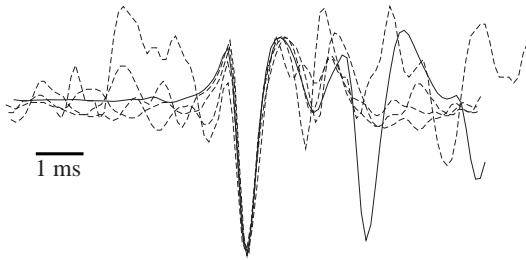


Fig. 7. Similarity in shape between single-unit spikes and a compound action potential (CAP) evoked by an intense 4.5 kHz stimulus. Dashed lines, spikes of four single receptors as they appear in the whole-nerve recording. Solid line, CAP evoked by a 4.5 kHz, 100 dB stimulus. Voltage scales differ for the different traces.

CAPs were edited with the program GoldWave before use in the model, so that they returned smoothly to the baseline.

Fig. 8 shows that, as expected, when model CAPs decrease in amplitude because of poor synchronization among receptors, they also increase in breadth (Fig. 8, open circles). As a crude test of the validity of the model, we analyzed the first three successive CAPs of responses to 4.5 kHz, 100 dB, which typically decrease progressively in amplitude (Figs 1, 2, 6). We assumed that the population of receptors participating in the response was relatively stable over this short period (approximately 10–12 ms) and, thus, that the decrease in

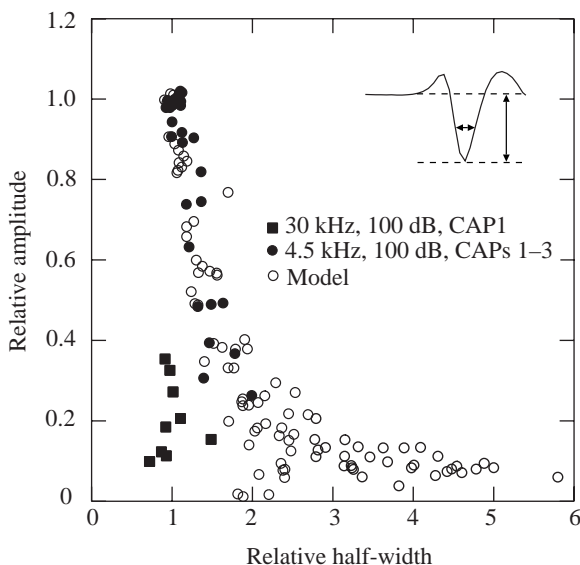


Fig. 8. The relationship between compound action potential (CAP) amplitude and CAP half-width. The graph includes results from nine crickets. The inset shows methods for measuring CAP amplitude and half-width (width at half-amplitude). Open circles, model CAPs produced by varying the degree of synchronization of the contributing receptors, normalized with respect to the amplitude and half-width of CAPs produced with perfect synchronization (see text for further explanation). Filled circles, first three successive CAPs of responses to 4.5 kHz, 100 dB, normalized with respect to the first CAP. Filled squares, first CAP of the response to 30 kHz, 100 dB, normalized with respect to the first CAP elicited by 4.5 kHz, 100 dB.

amplitude of successive CAPs was due to decreasing synchronization among receptors rather than to a decrease in the numbers of receptors responding. The points corresponding to these CAPs follow the prediction of the model rather closely (Fig. 8, filled circles). In contrast, CAPs evoked by 30 kHz, 100 dB, though small in amplitude, were considerably less broad than predicted in the model. We conclude that the small amplitudes of these CAPs cannot be accounted for by poor synchronization of receptors.

#### *Number of receptors contributing to CAPs at 4.5 kHz and 30 kHz*

For responses in which receptors are highly synchronized, i.e. at high stimulus intensity (Fig. 7), the number of receptors contributing to the CAP will be approximated by the ratio of the CAP amplitude to the amplitude of single-unit spikes. We calculated these ratios for responses to 100 dB stimuli for five preparations, using the mean amplitude of all single units recorded per preparation ( $N=1-5$ ) as the denominator. An average of  $14.6 \pm 2.6$  (mean  $\pm$  s.d.) receptors contribute to CAPs at 4.5 kHz, 100 dB, whereas only  $4.2 \pm 2.7$  contribute to 30 kHz CAPs ( $P < 0.001$ , paired  $t$ -test).

#### Discussion

Our results support the hypothesis that the two frequency ranges known to be behaviorally relevant to *T. oceanicus* are represented unequally at the level of auditory receptors. Compound action potentials are up to four times larger for stimuli at a frequency of 4.5 kHz than for those at 30 kHz (Fig. 2). This cannot be ascribed to differences in unit spike sizes or in the degree of synchronization of receptors that respond to these two frequencies, leaving different numbers of receptors as the most likely explanation. This conclusion supports Pollack's (1994) suggestion that central neurons are driven by a relatively large number of low-frequency receptors and by relatively few ultrasound receptors.

We used conduction velocity as an indirect measure of spike amplitude. Both of these parameters reflect longitudinal current flow during propagation of the action potential. The magnitude of the current depends on the change in membrane potential (i.e. the amplitude of the action potential as recorded intracellularly) and on the axial resistance of the axon which, in turn, depends on the resistivity of its axoplasm and on its cross-sectional area (Hodgkin, 1954; Rushton, 1951). Thus, although we found no difference in conduction velocity between low- and high-frequency receptors, this implies equal spike amplitudes only if intracellular action potential amplitude and axoplasmic resistivity are identical. Our conclusions from conduction-velocity measurements are supported by direct measurements of spike amplitude and suggest that these conditions are met.

Most of our measurements of conduction velocity were made from whole-nerve recordings, using the negative peak of the CAP to indicate its arrival time at each recording electrode. This is a rather poorly defined aggregate measure of the

conduction velocities of all of the receptors that contribute to the CAP. Conduction velocities were similar when measured for single units (Fig. 5B), suggesting that any errors associated with measurements from CAPs are minor. More importantly, both sorts of measurements indicated that conduction velocities are similar for low- and high-frequency receptors.

Pollack (1994) found that the response latency of the interneuron ON1 is longer for low-frequency stimuli than for ultrasonic stimuli and hypothesized that this might be due to more rapid spike conduction in high-frequency receptors. These receptors drive short-latency responses that are believed to mediate escape from predators (Hoy, 1991), and selection for high conduction velocity would not be unexpected. We can now reject this hypothesis; the explanation for the latency difference of ON1 must be sought elsewhere. Experiments toward this end are in progress (Faulkes and Pollack, 1997).

We measured the relationship between the amplitude and breadth of CAPs to determine whether differences in spike synchronization might explain the amplitude difference of CAPs in response to low and high frequencies. Ultrasound-elicited CAPs were not broader than those evoked by 4.5 kHz (Fig. 7), nor did their relative amplitude and breadth match the predictions of a model of the effect of synchronization on CAP amplitude (Fig. 8). We did find that successive CAPs within a single response (to 4.5 kHz), which might be expected to show increasingly poor synchronization, did match the prediction of the model (Fig. 8). This gives us some confidence that the model we used is an appropriate one.

Our measurements include only receptors whose spikes arrive at the recording electrode nearly synchronously. Receptors that respond to a stimulus, but whose latencies or conduction velocities differ from the 'norm', will be excluded. The low-frequency receptor of Fig. 3, for example, responded to 30 kHz stimuli, but with such long latency that its spike would not contribute to the first CAP of the response. The latency of this receptor to 30 kHz is approximately 10 ms longer than the latency of another receptor(s?) in the same preparation, visible as small deflections of the trace. CAPs are only 1–2 ms in duration (Fig. 6), so receptors will be excluded from the first CAP of a response even with much smaller latency shifts than that shown in Fig. 3. Single-unit recordings show that, whereas low-frequency receptors only rarely respond to ultrasound, most high-frequency receptors respond to 4–5 kHz stimuli if intensity is high enough, although usually with longer latency than to the frequency to which they are most sensitive (Imaizumi and Pollack, 1997, and in preparation). By comparing CAP amplitudes and single-unit amplitudes, we estimate that approximately 15 receptors contribute to the first CAP of the response evoked by 4.5 kHz, 100 dB. If the total number of receptors in the ear of *T. oceanicus* approximates that in the closely related species *T. commodus*, which has approximately 70 receptors (Young and Ball, 1974), then only approximately 20% of the receptor population is included in the first CAP evoked by this stimulus. This probably seriously underestimates the total number of receptors that respond to this stimulus and may more closely

reflect the number of receptors that are tuned to frequencies near 4.5 kHz, which would be expected to have the shortest latencies. A similar argument applies to the few receptors that contribute to CAPs elicited by 30 kHz, 100 dB stimuli.

Previous studies in crickets (summarized in Pollack, 1994), using single-unit recording techniques, also found that many receptors were tuned to the conspecific song frequency. In the single study that investigated sensitivity to ultrasound (Hutchings and Lewis, 1981), 36% of the recorded receptors were tuned to 4–5 kHz and at least 10% were tuned to ultrasound. That the agreement between these estimates (which are based on recordings from 42 receptors) and ours is imperfect is perhaps not surprising, given the differences in technique.

The representation of sound frequency at the receptor level has been studied in other orthopteran insects. In tettigoniids, the frequency-sensitivity of receptors is distributed more or less evenly throughout the hearing range (e.g. Römer, 1983; Kalmring *et al.* 1990), although the occurrence of several receptors with similar tuning (to the dominant frequency of the calling song) has been reported (Oldfield, 1984; H. Stölting and A. Stumpner, personal communication). The broad representation of sound frequency in tettigoniid ears corresponds to the rather broad-band acoustic signals of these insects (Kalmring *et al.* 1990). In acridids, receptors fall into four groups on the basis of both anatomy and physiology (Gray, 1960; Michelsen, 1971; Römer, 1976; Halex *et al.* 1988). Two of these groups, which share similar tuning to low frequencies, account for more than half the receptors in the ear (physiological types 1 and 2, anatomical groups a and c: Gray, 1960; Römer, 1976; Halex *et al.* 1988). Little is known about acoustic behavior in the species in which receptors have been studied most thoroughly (*Locusta migratoria*, *Schistocerca gregaria*), so the functional significance of this striking over-representation of a limited portion of the hearing range is not known. Experiments with another species, *Chorthippus biguttulus*, demonstrate that acridids do analyze the frequency content of acoustic signals. Males perform phonotaxis only in response to the low-frequency components of the broad-band signal of the female, while females answer male songs reliably only when both low- and high-frequency components of the song are present (von Helversen and von Helversen, 1997).

The best-studied example of peripheral over-representation of behaviorally relevant frequencies occurs in CF-FM bats, whose echolocation cries include a long constant-frequency (CF) tone. The region of the basilar membrane that is maximally excited by echoes of this CF frequency is expanded (Bruns, 1976), forming an 'acoustic fovea' (Suga and Jen, 1976). Receptors that are tuned to the CF range are disproportionately numerous and are also more sharply tuned than receptors for other frequencies (Suga *et al.* 1976). Over-representation of the CF range persists through central levels of auditory processing (Suga and Jen, 1976; Schuller and Pollak, 1979).

In crickets, sensitivity to 4–5 kHz and to ultrasound is apparent at the level of interneurons. Of six auditory

interneurons that are known or, on the basis of anatomy, are likely to receive direct input from auditory receptors (Wohlers and Huber, 1985; Atkins and Pollack, 1987a; Hennig, 1988), three are primarily sensitive to 4–5 kHz (AN1, DN1, TN1; Wohlers and Huber, 1982; Atkins and Pollack, 1987b), another is mainly sensitive to ultrasound (AN2; Moiseff and Hoy, 1983), one shows dual sensitivity to both 4–5 kHz and to ultrasound (ON1; Atkins and Pollack, 1986) and the sixth shows broad-band tuning (ON2; Wohlers and Huber, 1982). Auditory information is carried to the brain, where behavioral decisions are made (Pollack and Hoy, 1981; Schildberger, 1984; Schildberger and Hörner, 1988), mainly by two first-order ascending interneurons, one (AN1) tuned to 4–5 kHz and the other (AN2) to higher frequencies. Among second- and higher-order interneurons, sensitivity to either or both frequency ranges appears to be relatively common (Atkins and Pollack, 1987a,b; Boyan, 1980; Brodfuehrer and Hoy, 1990; Schildberger, 1984). It is evident that the numerical under-representation of ultrasound receptors does not prevent them from having strong influences on central neurons and on behavior (Pollack, 1994). In this respect, crickets are similar to moths, in which detection and evasion of echolocating bats can be accomplished with as few as a single receptor neuron per ear (Surlykke, 1984).

Even in the absence of marked central over-representation of low sound frequencies, an abundance of low-frequency receptors might enhance the processing of intraspecific signals. First, differences in threshold among receptors would be expected to increase the dynamic range of their summed input beyond that of any single receptor, perhaps accounting for the observed wide (>50 dB) dynamic range of phonotactic responses to cricket song (Pollack and Plourde, 1982). Second, central convergence of the activity of a number of receptors might allow more precise encoding of the temporal features of the stimulus (Carr *et al.* 1986), which are known to be important in triggering behavioral responses to cricket song (Pollack and Hoy, 1979; Thorson *et al.* 1982; Pollack and El-Feghaly, 1993). Synchronous activity of groups of receptors might also provide more accurate cues for sound direction. In acridids, interaural latency differences of receptors, generated by the acoustic directionality of the auditory system and the intensity-dependence of response latency, can serve as a cue for determining sound direction (Rheinlaender and Morchen, 1979; von Helversen and Rheinlaender, 1988), and preliminary experiments indicate that this may be true for crickets as well (D. von Helversen and G. S. Pollack, unpublished observations). Determining interaural latency difference from the responses of single receptors is problematic because of the variability of latency both within and between receptors. The concerted activity of a number of receptors, however, could provide a well defined time marker for response latency in each ear (Krahe and Ronacher, 1993). Moreover, because responses to single sound pulses consist of trains of regularly spaced CAPs (Figs 1, 2), central neurons might be able to make multiple comparisons of interaural latency, once for each CAP in the response.

This work was supported by grants from NSERC and the Whitehall Foundation.

## References

- ADAM, L.-J. (1977a). The oscillating summed action potential of an insect's auditory nerve (*Locusta migratoria*, Acrididae). I. Its original form and time constancy. *Biol. Cybernetics* **26**, 241–247.
- ADAM, L.-J. (1977b). The oscillating summed action potential of an insect's auditory nerve (*Locusta migratoria*, Acrididae). II. Underlying spike pattern and causes of spike synchronization. *Biol. Cybernetics* **28**, 109–119.
- ATKINS, G. AND POLLACK, G. S. (1986). Age dependent occurrence of an ascending axon on the omega neuron of the cricket, *Teleogryllus oceanicus*. *J. comp. Neurol.* **243**, 527–534.
- ATKINS, G. AND POLLACK, G. S. (1987a). Correlations between structure, topographic arrangement and spectral sensitivity of sound-sensitive interneurons in crickets. *J. comp. Neurol.* **266**, 398–412.
- ATKINS, G. AND POLLACK, G. S. (1987b). Response properties of prothoracic, interganglionic, sound-activated interneurons in the cricket *Teleogryllus oceanicus*. *J. comp. Physiol. A* **161**, 681–693.
- BALAKRISHNAN, R. AND POLLACK, G. S. (1996). Recognition of courtship song in the field cricket, *Teleogryllus oceanicus*. *Anim. Behav.* **51**, 353–366.
- BOYAN, G. S. (1980). Auditory neurones in the brain of the cricket *Gryllus bimaculatus* (DeGeer). *J. comp. Physiol. A* **140**, 81–93.
- BRODFUEHRER, P. D. AND HOY, R. R. (1990). Ultrasound sensitive neurons in the cricket brain. *J. comp. Physiol. A* **166**, 651–662.
- BRUNS, B. (1976). Peripheral auditory tuning for fine frequency analysis by the CF-FM bat, *Rhinolophus ferrumequinum*. II. Frequency mapping in the cochlea. *J. comp. Physiol. A* **106**, 87–97.
- CARR, C. E., HEILIGENBERG, W. AND ROSE, G. J. (1986). A time-comparison circuit in the electric fish midbrain. I. Behavior and physiology. *J. Neurosci.* **6**, 107–119.
- EIBL, E. AND HUBER, F. (1979). Central projections of tibial sensory fibers within the three thoracic ganglia of crickets (*Gryllus campestris* L., *Gryllus bimaculatus* DeGeer). *Zoomorphologie* **92**, 1–17.
- FAULKES, Z. AND POLLACK, G. S. (1997). Sound frequency specific responses of cricket omega neuron. *Soc. Neurosci. Abstr.* **23**, 1570.
- FULLARD, J. H., MORRIS, G. K. AND MASON, A. C. (1989). Auditory processing in the black-sided meadow katydid *Conocephalus nigropleurum* (Orthoptera: Tettigoniidae). *J. comp. Physiol. A* **164**, 501–512.
- GRAY, E. G. (1960). The fine structure of the insect ear. *Phil. Trans. R. Soc. Lond. B* **243**, 75–94.
- HALEX, H., KAISER, W. AND KALMRING, K. (1988). Projection areas and branching patterns of the tympanal receptor cells in migratory locusts, *Locusta migratoria* and *Schistocerca gregaria*. *Cell Tissue Res.* **253**, 517–528.
- HENNIG, R. M. (1988). Ascending auditory interneurons in the cricket *Teleogryllus commodus* (Walker): comparative physiology and direct connections with afferents. *J. comp. Physiol. A* **163**, 135–143.
- HODGKIN, A. L. (1954). A note on conduction velocity. *J. Physiol., Lond.* **125**, 221–224.
- HOY, R. R. (1991). Signals for survival in the lives of crickets. *Am. Zool.* **31**, 169–185.
- HOY, R. R. AND ROBERT, D. (1996). Tympanal hearing in insects. *A. Rev. Ent.* **41**, 433–450.



- HUBER, F., WOHLERS, D. W. AND MOORE, T. E. (1980). Auditory nerve and interneurone responses to natural sounds in several species of cicadas. *Physiol. Ent.* **5**, 25–45.
- HUTCHINGS, M. AND LEWIS, B. (1981). Response properties of primary auditory fibers in the cricket *Teleogryllus oceanicus* (Le Guillou). *J. comp. Physiol. A* **143**, 129–134.
- IMAIZUMI, K. AND POLLACK, G. S. (1997). Physiological properties of auditory receptors in the Australian field cricket *Teleogryllus oceanicus*. *Soc. Neurosci. Abstr.* **23**, 1571.
- KALMRING, K., KEUPER, A. AND KAISER, W. (1990). Aspects of acoustic and vibratory communication in seven European bushcrickets. In *The Tettigoniidae: Biology, Systematics and Evolution* (ed. W. J. Bailey and D. C. Rentz), pp. 191–216. Berlin: Springer-Verlag.
- KRAHE, R. AND RONACHER, B. (1993). Long rise times of sound pulses in grasshopper songs improve the directionality cues received by the CNS from the auditory receptors. *J. comp. Physiol. A* **173**, 425–434.
- MICHELSSEN, A. (1971). The physiology of the locust ear. I. Frequency sensitivity of single cells in the isolated ear. *Z. vergl. Physiol.* **71**, 49–62.
- MOISEFF, A. AND HOY, R. R. (1983). Sensitivity to ultrasound in an identified auditory interneuron in the cricket: a possible neural link to phonotactic behavior. *J. comp. Physiol. A* **131**, 113–120.
- MOISEFF, A., POLLACK, G. S. AND HOY, R. R. (1978). Steering responses of flying crickets to sound and ultrasound: mate attraction and predator avoidance. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4052–4056.
- OLDFIELD, B. P. (1984). Physiology of auditory receptors in two species of Tettigoniidae (Orthoptera: Ensifera). *J. comp. Physiol. A* **155**, 689–696.
- PEARSON, K. G., STEIN, R. B. AND MALHOTRA, S. K. (1970). Properties of action potentials from insect motor nerve fibres. *J. exp. Biol.* **53**, 299–316.
- POLLACK, G. S. (1994). Synaptic inputs to the omega neuron of the cricket *Teleogryllus oceanicus*: differences in EPSP waveforms evoked by low and high sound frequencies. *J. comp. Physiol. A* **174**, 83–89.
- POLLACK, G. S. (1997). SWEEPS: a program for the acquisition and analysis of neurophysiological data. *Comp. Meth. Prog. Biomed.* **53**, 163–173.
- POLLACK, G. S. AND EL-FEGHALY, E. (1993). Calling song recognition in the cricket *Teleogryllus oceanicus*: comparison of the effects of stimulus intensity and sound spectrum on selectivity for temporal pattern. *J. Comp. Physiol. A* **171**, 759–765.
- POLLACK, G. S. AND HOY, R. R. (1979). Temporal pattern as a cue for species-specific calling song recognition in crickets. *Science* **204**, 423–432.
- POLLACK, G. S. AND HOY, R. R. (1981). Phonotaxis in flying crickets: neural correlates. *J. Insect Physiol.* **27**, 41–45.
- POLLACK, G. S. AND PLOURDE, N. (1982). Directionality of acoustic orientation in flying crickets. *J. comp. Physiol. A* **146**, 207–215.
- RHEINLAENDER, J. AND MORCHEN, A. (1979). ‘Time–intensity trading’ in locust auditory interneurons. *Nature* **281**, 672–674.
- RÖMER, H. (1976). Die Informationsbearbeitung tympanaler Rezeptorelemente von *Locusta migratoria* (Acrididae, Orthoptera). *J. comp. Physiol.* **109**, 101–122.
- RÖMER, H. (1983). Tonotopic organization of the auditory neuropile in the bushcricket *Tettigonia viridissima*. *Nature* **306**, 60–62.
- RÖSSLER, W., BAILEY, W. J., SCHRÖDER, J. AND KALMRING, K. (1990). Resolution of time and frequency patterns in the tympanal organs of tettigoniids. I. Synchronization and oscillation in the activity of receptor populations. *Zool. Jb. Physiol.* **94**, 83–99.
- RUSHTON, W. A. H. (1951). A theory of the effects of fibre size in medullated nerve. *J. Physiol., Lond.* **115**, 101–122.
- SCHILDBERGER, K. (1984). Temporal selectivity of identified auditory neurons in the cricket brain. *J. comp. Physiol. A* **155**, 171–185.
- SCHILDBERGER, K. AND HÖRNER, M. (1988). The function of auditory neurons in cricket phonotaxis. I. Influence of hyperpolarization of identified neurons on sound localization. *J. comp. Physiol.* **163**, 621–632.
- SCHULLER, G. AND POLLACK, G. (1979). Disproportionate frequency representation in the inferior colliculus of Doppler-compensating greater horseshoe bats: evidence for an acoustic fovea. *J. comp. Physiol. A* **132**, 47–54.
- SEARLE, S. R. (1987). *Linear Models for Unbalanced Data*. New York: Wiley.
- SHAW, S. R. (1994). Detection of airborne sound by a cockroach ‘vibration detector’: a possible missing link in insect auditory evolution. *J. exp. Biol.* **193**, 13–47.
- SIMMONS, J. A., FENTON, M. B. AND O’FARRELL, M. J. (1979). Echolocation and pursuit of prey by bats. *Science* **203**, 16–21.
- STRAUSFELD, N. J., SEYAN, H. S., WOHLERS, D. AND BACON, J. P. (1983). Lucifer Yellow histology. In *Functional Neuroanatomy* (ed. N. J. Strausfeld), pp. 132–155. Berlin: Springer-Verlag.
- SUGA, N. AND JEN, P. H. S. (1976). Disproportionate tonotopic representation for processing CF-FM signals in the mustache bat auditory cortex. *Science* **194**, 542–544.
- SUGA, N., NEUWEILER, G. AND MÖLLER, J. (1976). Peripheral auditory tuning for fine frequency analysis of the CF-FM bat, *Rhinolophus ferrumequinum*. IV. Properties of peripheral auditory neurons. *J. comp. Physiol.* **106**, 111–125.
- SURLYKKE, A. (1984). Hearing in notodontid moths: a tympanic organ with a single auditory neurone. *J. exp. Biol.* **113**, 323–335.
- THORSON, J., WEBER, T. AND HUBER, F. (1982). Auditory behavior of the cricket. II. Simplicity of calling-song recognition in *Gryllus* and anomalous phonotaxis at abnormal carrier frequencies. *J. comp. Physiol. A* **146**, 361–378.
- VON HELVERSEN, D. AND RHEINLAENDER, J. (1988). Interaural intensity and time discrimination in an unrestrained grasshopper: a tentative behavioural approach. *J. comp. Physiol. A* **162**, 333–340.
- VON HELVERSEN, D. AND VON HELVERSEN, O. (1997). Recognition of sex in the acoustic communication of the grasshopper *Chorthippus bigguttulus* (Orthoptera, Acrididae). *J. comp. Physiol. A* **180**, 373–386.
- WOHLERS, D. W. AND HUBER, F. (1982). Processing of sound signals by six types of neurons in the prothoracic ganglion of the cricket, *Gryllus campestris* L. *J. comp. Physiol. A* **146**, 161–173.
- WOHLERS, D. W. AND HUBER, F. (1985). Topographical organization of the auditory pathway within the prothoracic ganglion of the cricket *Gryllus campestris* L. *Cell Tissue Res.* **239**, 555–565.
- YAGER, D. D. AND SPANGLER, H. G. (1995). Characterization of auditory afferents in the tiger beetle, *Cicindela marutha* Dow. *J. comp. Physiol. A* **176**, 587–599.
- YOUNG, D. AND BALL, E. (1974). Structure and development of the auditory system in the prothoracic leg of the cricket *Teleogryllus commodus* (Walker). *Z. Zellforsch.* **147**, 293–312.