Intracellular Responses of Antennal Chordotonal Sensilla of the American Cockroach

Authors: Ikeda, Suguru, Toh, Yoshihiro, Okamura, Jun-ya, and Okada, Jiro

Source: Zoological Science, 21(4) : 375-383

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.21.375

ZOOLOGICAL SCIENCE **21**: 375–383 (2004)

Intracellular Responses of Antennal Chordotonal Sensilla of the American Cockroach

Suguru Ikeda, Yoshihiro Toh*, Jun-ya Okamura and Jiro Okada

Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812-8581, Japan

ABSTRACT—The responses of mechanoreceptor neurons in the antennal chordotonal organ have been examined in cockroaches by intracellular recording methods. The chordotonal organ was mechanically stimulated by sinusoidal movement of the flagellum. Stimulus frequencies were varied between 0.5 and 150 Hz. Receptor neurons responded with spike discharges to mechanical stimulation, and were classed into two groups from plots of their average spike frequencies against stimulus frequency. Neurons in one group responded to stimulation over a wide frequency range (from 0.5 to 150 Hz), whereas those in a second group were tuned to higher frequency stimuli. The peak stimulus frequency at which receptor neurons showed maximum responses differed from cell to cell. Some had a peak response at a stimulus frequency given in the present study (from 0.5 to 150 Hz), whereas others were assumed to have peak responses beyond the highest stimulus frequency examined.

The timing for the initiation of spikes or of a burst of spikes plotted against each stimulus cycle revealed that spike generation was phase-locked in most cells. Some cells showed phase-independent discharges to stimulation at lower frequency, but increasing stimulus frequencies spike initiation began to assemble at a given phase of the stimulus cycle. The response patterns observed are discussed in relation to the primary process of mechanoreception of the chordotonal organ.

Key words: cockroach, antenna, chordotonal organ, mechanoreceptor, intracellular recording

INTRODUCTION

The insect antenna is a multi-modal sense organ equipped with olfactory, gustatory, tactile, temperature and humidity receptors. Since the insect detects the direction of a stimulus source using its antennal receptors, it has to monitor the position and direction of movement of its antennae. Some nocturnal walking insects such as American cockroaches grasp their surroundings largely depending on sensory reception from their antennae (e.g., Toh, 1977, Okada and Toh, 2000). A cockroach waves its antennae continually to scan the surrounding space. Therefore, monitoring the position and movement direction of the antennae from moment to moment must be important for such insects to specify reliably the direction or position of a stimulus source. Mechanoreceptors such as campaniform sensilla, hair plates, Johnston's organ and chordotonal organ occur in the basal part of the insect antenna, which undoubtedly play proprioceptive roles in the detection of antennal position and movement (e.g., Toh, 1981, Toh and Yokohari, 1985, Okada and Toh, 2000, 2001). In order to understand how each of these mechanoreceptors contributes to monitoring the antennal movement, its response properties need to be examined electrophysiologically. Since hair plate sensilla and campaniform sensilla are externally visible, they are accessible to electrophysiological techniques (e.g., Heinzel and Gewecke, 1979). On the other hand, electrophysiological approaches to Johnston's organ and the chordotonal organ are limited, because these are internal mechanoreceptors. Mass electrical activity has been reported in Johnston's organ of the mosquito (Göpfert and Robert, 2001), but the responses of individual receptor cells have not been recorded.

In the present study the responses of antennal chordotonal sensilla have been recorded intracellularly from receptor axons in American cockroaches. Chordotonal sensilla are widely distributed in various parts of the insect appendage. Their functions vary depending upon where they occur and what accessory structures they are equipped with (e.g., sound reception in the tympanal organ, vibration reception in the subgenual organ, proprioception in various segmental joints). There have been many electrophysiological reports of the responses of chordotonal sensilla in the tympanal organ, subgenual organ and various segmental joints (extensively reviewed by Field and Matheson, 1998), but

* Corresponding author: Tel. +81-92-642-2611;
FAX. +81-92-642-2315.
E-mail: yotohscb@mbox.nc.kyushu-u.ac.jp

few reports for the antennal chordotonal organ in any insect.

The present study is aimed at dealing with the response properties of the chordotonal organ of the insect antenna, with special reference to the discharge patterns of receptor neurons to sinusoidal mechanical stimulation. The data obtained are discussed in relation to the primary process of mechanoreception and in reference to the specific structures previously reported for the cockroach antennal chordotonal organ (Toh and Yokohari, 1985).

MATERIALS AND METHODS

Male adult cockroaches (*Periplaneta americana*) from a laboratory culture were used throughout the present work. The chordotonal organ is an elongated structure occurring in the hemolymph cavity of the antennal pedicel. Its basal region housing the receptor neurons is located in the equator region of the proximal part of the pedicel and ventrally its distal end joins the distal part of the pedicel (Fig. 1A). Receptor axons extend proximally as two bundles. The antennal nerve runs ventrally along the entire length of the antennal cavity, as two bundles, from the scape to the flagellum. The left and right bundles of chordotonal organ axons merge with the left and right bundles, respectively, of the antennal nerve in the scape. The pedicel-scape joint is flexible in a vertical plane, when the organism is in a normal standing posture. Thus, a dorso-ventral movement of the antennal flagellum mechanically stimulates the chordotonal sensilla.

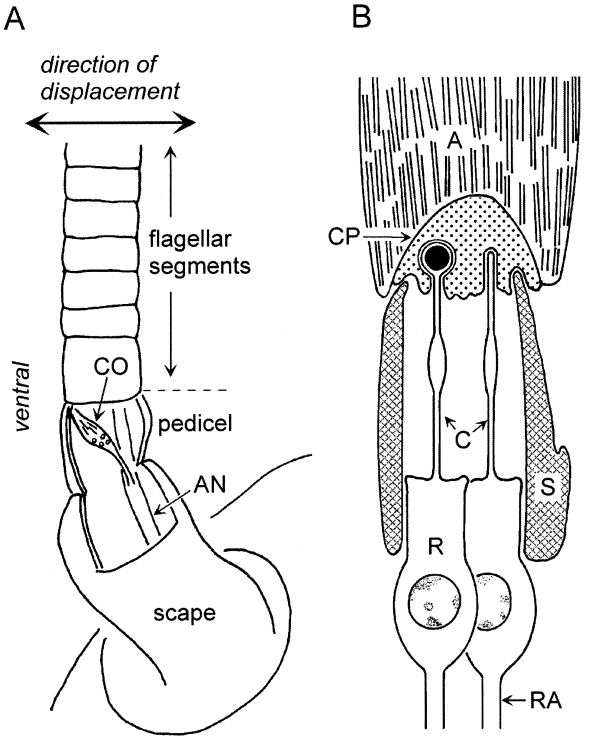

Fig. 1. **A** Drawing of a basal part of the antenna of the cockroach. A part of the cuticle in scape and pedicel was removed to expose chordotonal organ (CO) and an antennal nerve (AN). Of more than 100 flagellar segments only several are drawn. **B** Schematic drawing of a chordotonal sensillum modified from Toh and Yokohari (1985). Note different terminal profiles between the two sensory cilia (C). A, attachment cell; CP, cap; R, receptor cell; RA, receptor axon; S, scolopale cell

In the present study, a cockroach was waxed on an acrylic platform with the frontal surface of the head up so that the scape-pedicel was displaced in a horizontal plane. The head and the scape were immobilized by beeswax. A part of the cuticle of the scape was removed to expose the chordotonal nerves, and the nerves were desheathed enzymatically. For intracellular recording a glass microelectrode, with a 70–100 MΩ with tip resistance when filled with 3M KCl, was inserted into one of the chordotonal nerves near its origin from the chordotonal organ. An indifferent electrode was placed in the saline solution.

A galvanometer from the pen of a chart-recorder was used as a device for mechanical stimulation. The pen shaft was replaced with a thin acrylic rod (4 mm in diameter and 30 mm in length). One end of the rod was firmly fixed to the axis of the galvanometer, and the other end, the tip, was excavated to house the antenna. The distal part of the antennal flagellum was cut off leaving about 15 mm of its base. The truncated antenna was then inserted about 7 mm into the cannula of the rod. The axis of the pedicel was elevated dorsally by 20° with respect to that of the scape, at the resting position shown in Fig. 1A. The rod was displaced sinusoidally in the horizontal plane from the resting position by driving the galvanometer with a function generator at frequencies ranging from 0.5 Hz to 150 Hz (arrows in Fig. 1A). The relation between the current applied to the galvanometer and the amplitude of the displacement of the rod was calibrated in advance of each experiment. The displacement of the antenna, when measured at the tip of the rod, was adjusted to approximately 100 μm for most recordings, but in some recordings it was changed between 100 μm and 400 μm. The movement of the rod was monitored by a photosensor. An LED placed above the antenna was directed towards the end of a fiber optic bundle placed under the antenna. The other end of the fiber optic faced the photosensor placed away from the experimental set. The path of the light emitted from the LED was partially shaded when the antenna moved. The resultant changes in light intensity transmitted by the fiber optic were detected by a photosensor. Although a sinusoidal movement of the antenna did not result in sinusoidal modulation of the output of the photosensor, the output was sufficient to reveal when the antenna was at its dorsalmost and ventralmost position.

Responses were amplified through a DC amplifier and displayed on an oscilloscope. Data were stored on magnetic tapes for off-line analysis by a computer equipped with an analog-digital converter (CED, Micro1401). In order to see a correlation between the time of spike initiation and the phase of the sinusoidal stimulus cycle, data were analyzed using Spike 2 (CED). The stimulus cycle of 360° was divided into 30 bins, and histograms for the number of spikes accumulated in the 30 bins during repeated sinusoidal stimulation cycles were obtained. The distribution of total spike numbers was statistically evaluated by a Kolmogorov-Smirnov test.

RESULTS

Structure

The structure of the antennal chordotonal organ of the American cockroach has been reported in detail (Toh and Yokohari, 1985), but is briefly introduced here in relation to the present study. The antennal chordotonal organ consists of approximately fifty chordotonal sensilla. Each sensillum contains two receptor neurons, which occur in the mid- to proximal region of the pedicel (Fig. 1B). Each receptor neuron possesses a distal dendrite and a proximal axon. The dendrite gives rise to a single sensory cilium. A pair of cilia extends distally into a lumen formed by a scolopale cell and terminates in a cap structure. The scolopale cell is charac-

terized by electron-dense structures called scolopale rods that surround the lumen. The cap is also an electron-dense structure formed by extracellular materials. The attachment cell, which holds the cap, is a conical cell extending distally to join the ventral hypodermis of the distal region of the pedicel. The above mentioned structures are common to all

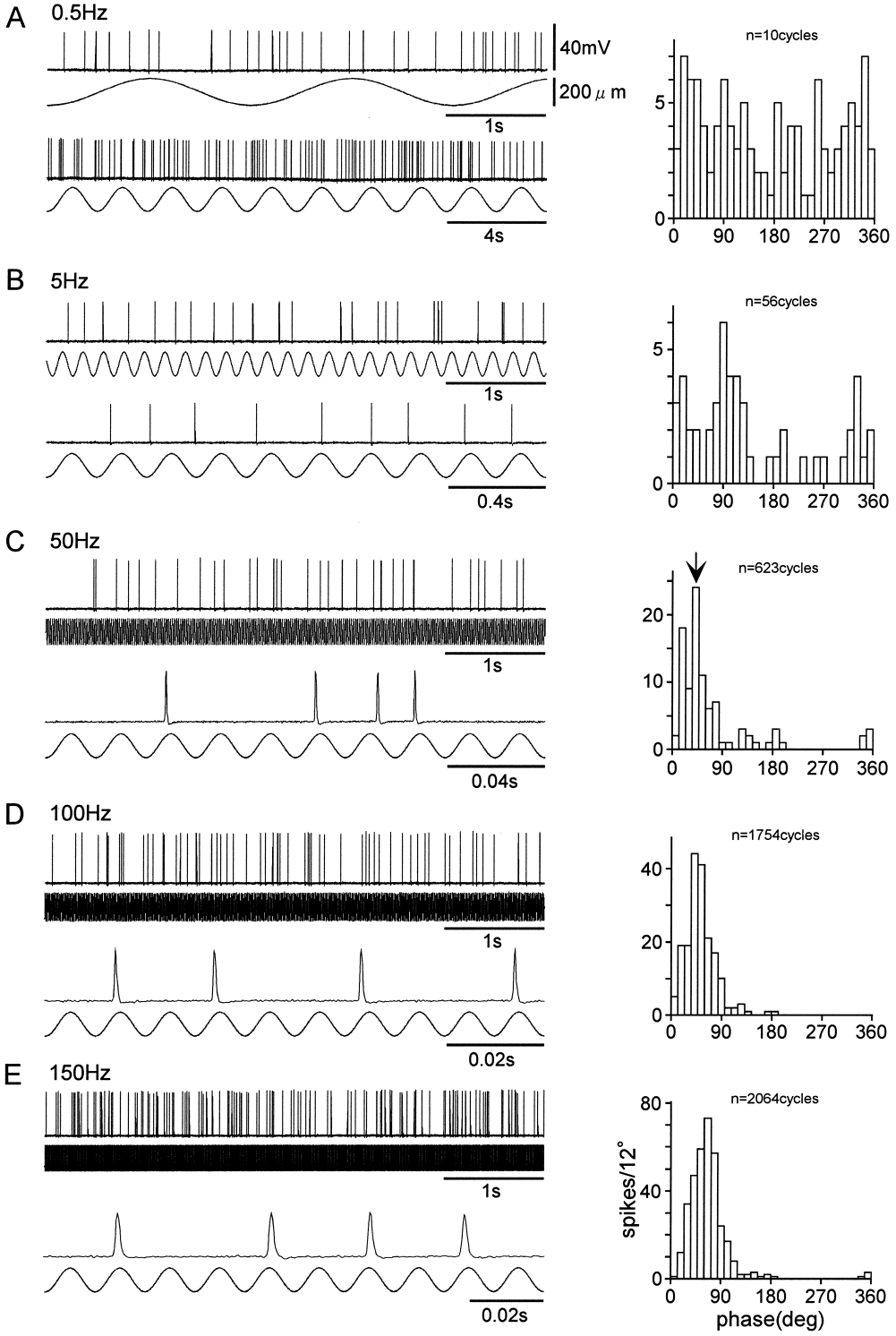

Fig. 2. Responses of a receptor cell to sinusoidal flagellar movements at five different frequencies (A, 0.5 Hz; B, 5 Hz; C, 50 Hz; D, 100 Hz; E, 150 Hz). Responses are shown throughout all stimulus frequencies with the same time scale (upper recordings) and for 10 stimulus cycles (lower recordings). Mechanical displacement of the antenna monitored by photosensor is given below each recording. In the histograms on the right the stimulus cycle is divided by every 12° into 30 phases, and the number of spikes for each bin is summated during repeated sinusoidal stimulation. The number of cycles for summation is given by *n* in each histogram. An arrow in C indicates a mode, which is obtained for Fig. 4.

insect chordotonal sensilla. A specific feature of the chordotonal sensilla in the present study is provided by the termination of the two cilia. These are both approximately 0.2 μm in diameter beneath the cap. One is enlarged up to a diameter of 0.7 μm within the cap, however, and contains an electron-dense structure (left cilium in Fig. 1B). Since the canal in the cap is narrow at its entrance and expands inside to hold the swollen part of the cilium, the terminal appears to be trapped in a bottleneck. The other cilium terminates without swelling (right cilium in Fig. 1B). Receptor axons

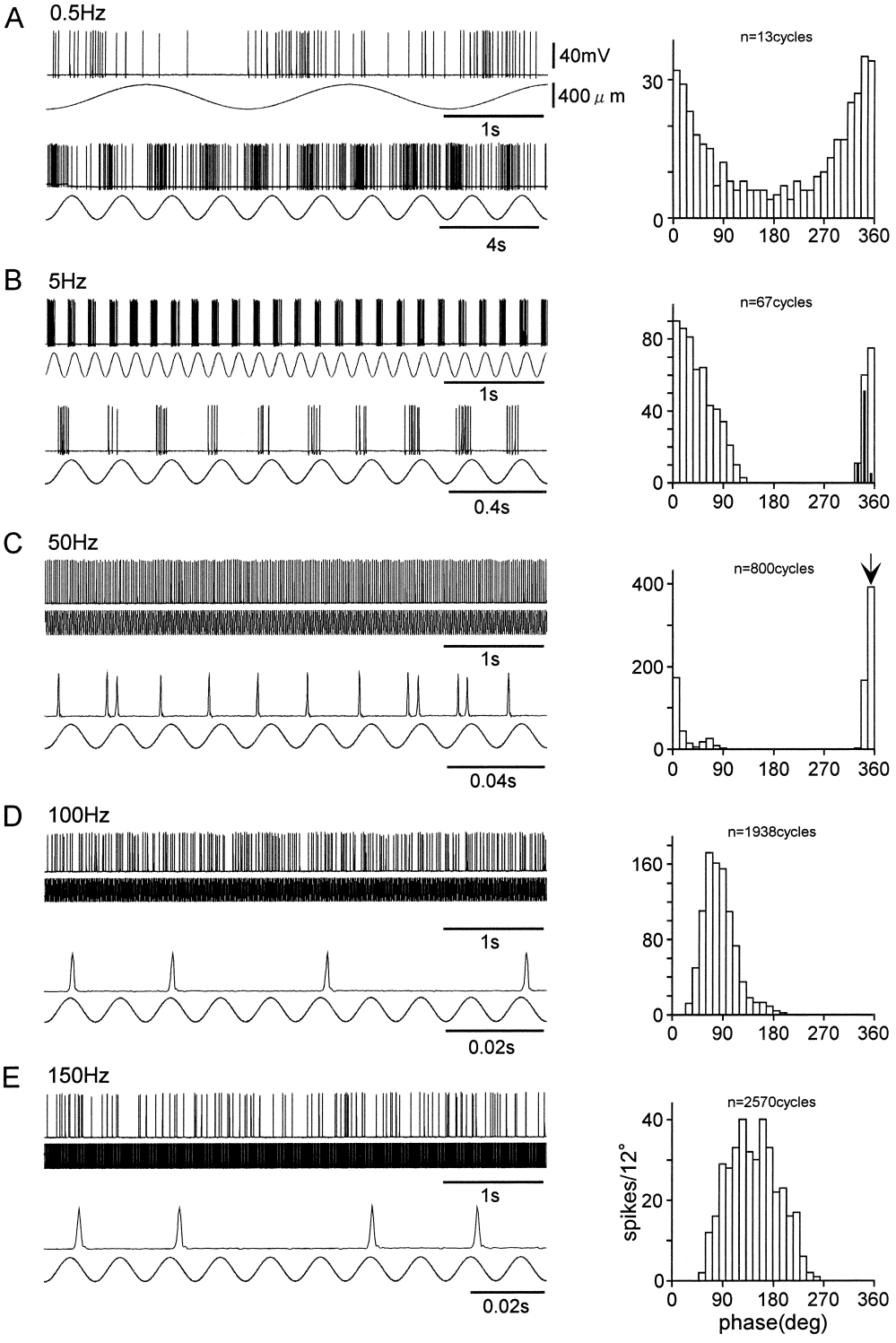

Fig. 3. Responses of another receptor cell to sinusoidal flagellar movements at five different frequencies (A, 0.5 Hz; B, 5 Hz; C, 50 Hz; D, 100 Hz; E, 150 Hz). In histogram in B the number of the first spikes of burst discharges are counted and expressed by solid column. Other labels are the same as those in Fig. 2.

from chordotonal sensilla are bundled as right and left chordotonal nerves. The thick antennal nerve running dorsally in the antennal cavity is also separated into right and left bundles. The right chordotonal nerve joins the right bundle of the antennal nerve, and the left joins the left bundle of the antennal nerve, in the distal part of the scape.

Responses to sinusoidal displacement of the flagellum

When a receptor axon was impaled by a microelectrode, a resting potential between –20 mV and –50 mV appeared. Some axons showed spontaneous spike activity up to a firing frequency of 25 Hz in the resting position of the scape-pedicel joint, but others were silent. In response to sinusoidal displacement of the flagellum spikes were elicited or their discharge frequencies were increased. Spike responses were often superimposed on graded potentials, but only the changes in discharge patterns are dealt with in this report. The flagellum was displaced with stimulus frequencies ranging from 0.5 Hz to 150 Hz. In the following description the responses will be evaluated by two different parameters, the phase at which spikes were initiated for each stimulus cycle, and the average spike frequency. The average spike frequency was obtained by dividing by 10 the total number of spikes occurring in a stimulus duration of ten seconds, irrespective of the pattern of the spike discharge.

Patterns of spike discharge at each stimulus cycle

In most responses spikes were elicited at a specific phase of the sinusoidal stimulus cycle, whereas in a small number of responses spikes were generated having no relation to the phase of the stimulus cycle. The distribution of the timing of spike discharges was analyzed quantitatively using phase histograms of spike generation. A stimulus cycle of 360° was divided into 30 equal bins in the histogram, and the total numbers of spikes elicited during repetitive sinusoidal stimulation shown for each bin (right columns in Figs. 2 and 3). The distribution of summed spike numbers over the 30 bins was tested statistically. When such numbers of spikes were significantly more frequent around a given phase than at other times (Kormogorov-Smirnov test; $P<0.05$), the response was referred to as phase-locked. Otherwise, when the distribution was less significant ($P>0.05$), the response was referred to as phase-independent. The frequencies of spike generation were rather evenly distributed in the histogram over all 30 bins in phase-independent discharges (Fig. 2A), whereas they were localized to several neighboring bins in phase-locked responses (Figs. 2B–E, Figs. 3A–E). In some phase-locked responses the histogram had a sharp peak, suggesting that discharges were highly synchronized (e.g., Fig. 3C). In others, it had a broader peak with a wider base. The wide base seemed to originate from several different factors. First, the spike discharges were loosely synchronized to the stimulus phase (e.g., Fig. 3A). Second, the spikes were highly synchronized to the stimulus phase, but occurred in bursts (e.g., Fig. 3B). If the timing of the first spike in the burst was plotted against the phase, a sharp peak appeared in this type of response (filled columns in Fig. 3B). Third, small fluctuations in the timing of phase-locked spikes at high frequency stimuli must necessarily increase the deviation in the phase of spike generation (e.g., Fig. 3E).

For descriptive convenience, phase-locked responses were classed into two subtypes. When spikes or bursts of spikes were elicited in phase in more than 80% of the stimulus cycles (Fig. 3B), such discharges were referred to as “tightly phase-locked responses”. On the other hand, when spikes were phase-locked but failed to occur in more than 20% of the stimulus cycles (Figs. 2C and 3C), such responses were referred to as “loosely phase-locked discharges”.

A given receptor neuron usually changed its discharge patterns as a function of stimulus frequency as shown in Figs. 2 and 3. The occurrence of each of the three discharge patterns specified above was counted without specifying the individual receptor neurons and the result is presented in Table 1. Table 1 shows a general tendency for the relationship between stimulus frequency and phase dependency of spike generation as follows. In response to stimulation at lower frequencies receptor neurons were apt to respond with phase-independent discharges as shown in Fig. 2A. Approximately 80% of the recorded cells showed such discharges to stimulation at 0.5 Hz. When stimulus frequencies were increased, however, the ratio of phase-independent discharges was decreased, whereas that of phase-locked discharges was increased, as shown in Figs. 2B, 2C, 3B, and 3C. In response to stimulation at 100 Hz only two cells

Table 1. Distribution of three types of discharge patterns together with no discharges in response to stimuli at five different frequencies.

stimulus frequency(Hz)	discharge pattern			
	tightly phase-locked	loosely phase-locked	phase-independent	no discharge
0.5 Hz (n=101)	10	3	50	28
5 Hz (n=107)	26	19	24	38
50 Hz (n=117)	38	50	7	22
100 Hz (n=95)	33	55	2	5
150 Hz (n=88)	22	57	1	8

showed phase-independent discharges, whereas 88 neurons showed phase-locked discharges. Moreover, the ratio of loosely phase-locked responses was increased when the stimulus frequency was increased above 50 Hz. Such a decrease in phase-locking seems to result from an increase in the number of stimulus cycles that failed to trigger spikes at high stimulus frequencies, as shown in Figs. 2D, 2E, 3D, and 3E.

The phase at which spikes were elicited did not shift greatly in individual neurons when stimulus frequency was changed, although it was advanced or delayed slightly (Figs. 2 and 3). The phase in the stimulus cycle which triggered spikes at the highest frequency, referred to as the mode phase, differed from cell to cell. Phase histograms for spike generation to stimulation at 50 Hz were obtained in 88 distinct phase-locked cells, and a mode phase (the bin at the mode) was obtained for each of the corresponding 88 histograms. The mode phases appeared to be widely distributed throughout the cycle (0°–360°), but their frequencies were low around 90° and 270°, as shown in Fig. 4 ($P<0.05$, Kormogorov-Smirnov test). It is assumed that cells that discharge spikes around the dorsal and ventral turning positions of the displacement are less frequent, since the antenna is changing its movement direction at phases 90° and 270°.

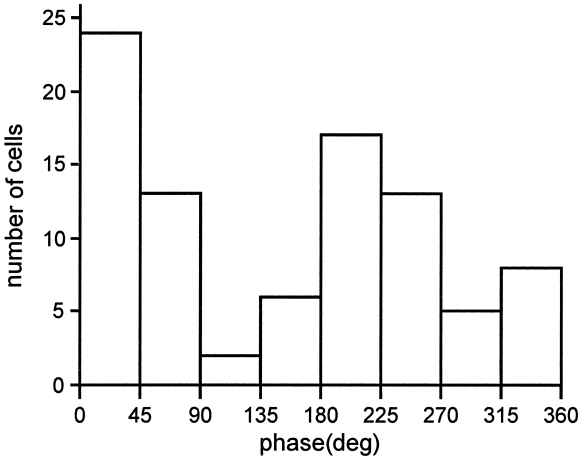

Fig. 4. Distribution of phases at which the cumulated number of spikes are the largest to stimulation at 50 Hz in phase-locked responses of 88 cells. The sinusoidal cycle is divided by every 45° into 8 phases (bins). A mode bin is specified in each phase histogram as shown by an arrow in Figs. 2C and 3C. Mode phases from 88 responses are summated in this histogram.

Changes in the average spike frequency (number of spikes per unit time)

From plots of their average spike frequency against stimulus frequency, receptor neurons were classed into two types, wide-frequency tuned cells and high-frequency tuned cells. The former responded to mechanical stimulation over a wide range of frequencies from 0.5 Hz to more than 100 Hz (Fig. 5A), whereas the latter responded only to relatively high frequencies above 50 Hz (Fig. 5B). Of 128 neurons examined 79 were wide-frequency tuned cells, and 49 were high-frequency tuned cells.

Receptor neurons were also classed into two types by the shapes of their frequency-response curves. In one type the frequency-response curve had a peak within the frequency range used in the present study (cells labeled by a cross, circle or diamond in Fig. 5A, and by a cross and square symbol in Fig. 5B), whereas in others the responses continued to increase with increased frequency without showing a peak response in the frequency range used (cells labeled by an open circle and diamond symbol in Fig. 5B). There does not seem to be basic difference between the two types, however, rather the difference may be due simply to the limitations of the stimulation system. If stimulus frequencies had been further increased beyond 150 Hz, the aver-

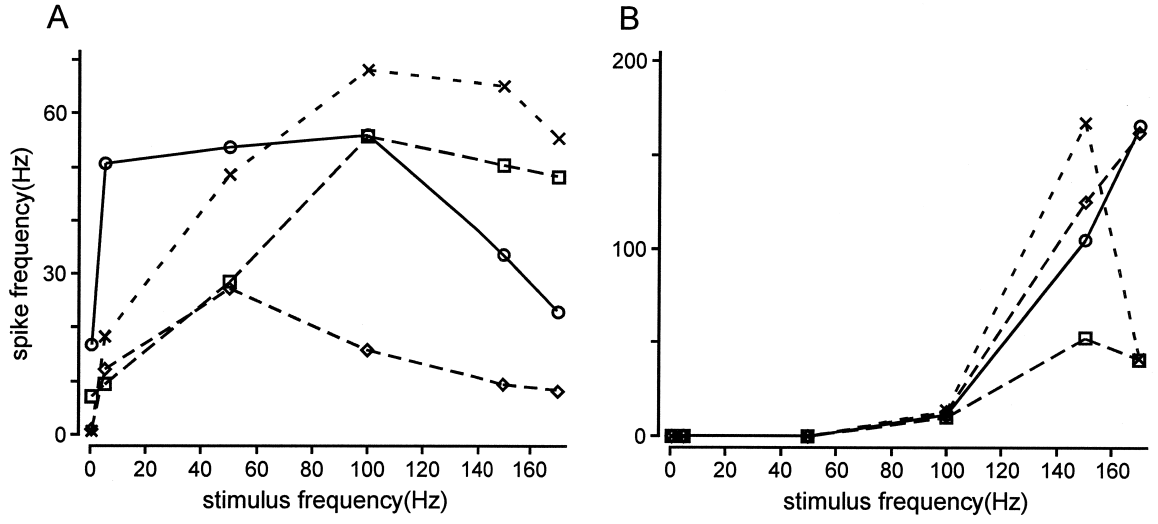

Fig. 5. Spike frequencies of receptor cells plotted against stimulus frequencies. A: four wide-frequency tuned cells. B: four high-frequency tuned cells.

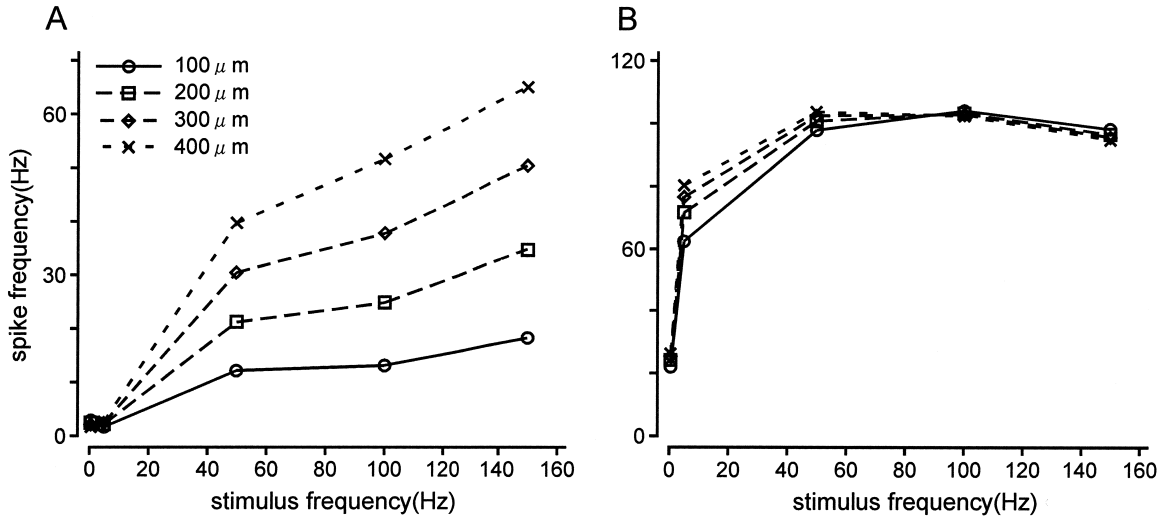

Fig. 6. Spike frequencies of receptor cells plotted against stimulus frequencies at four different amplitudes of the antennal displacement. In four responses of a cell shown in **A** stimulus frequency-response curves become steep when the stimulus amplitude is increased. In four responses of another cell shown in **B** frequency-response curves are affected little by change in stimulus amplitude.

age spike frequencies of the latter group would have dropped below their peak responses to some higher frequency stimuli. Therefore, one difference between the two types of neuron is at what stimulus frequency the neuron shows a peak response.

The shape of the frequency-response curve depends on how the number of spikes for each stimulus cycle changes with increases in the stimulus frequency. In a stimulus frequency range in which the number of spikes elicited for every cycle is nearly the same, the average spike frequency increases linearly with stimulus frequency as a simple consequence of the increasing number of stimulus cycles included in unit time. Such linear increase frequently occurred in response to stimulation from a low (0.5 Hz) to a medium frequency (e.g., 50 Hz). In a stimulus frequency range in which the number of spikes elicited for every cycle increased with stimulus frequency, the average spike frequencies were steeply increased as a function of the increase in stimulus frequency. In a stimulus frequency range at which the number of spikes elicited for every cycle decreases, or there was an increased probability of failure to discharge, the average spike frequencies increased slowly as a function of increasing stimulus frequency, or attained a plateau. When the probability of failure to discharge further increased in response to stimulation at a yet higher frequency, the average spike frequency decreased after a peak response, as shown in Fig. 3. The decrease in average spike frequency seen from C to E in Fig. 3 is thus due to such increases in the probability of a failure to discharge.

Responses to changing amplitude of displacement of the flagellum

Sinusoidal mechanical stimulation was applied using amplitudes of from 100 μm to 400 μm in some specimens. In most neurons (37 of 41 examined) the average spike frequencies were increased when the stimulus amplitude was increased (Fig. 6A). The response curve plotted as a function of stimulus frequency was shifted upward when the amplitude was increased in those cells. However, a few neurons (4 out of 41) changed their discharge frequencies little when the stimulus amplitude was changed between 100 μm and 400 μm (Fig. 6B).

DISCUSSION

In the present study the responses of receptor neurons in antennal chordotonal sensilla to mechanical displacement have been examined from intracellular recordings. The antennal flagellum was displaced sinusoidally at frequencies from 0.5 to 150 Hz. Since under in the natural habitat American cockroaches spontaneously wave their antennae at a frequency less than10 Hz, it is unlikely that the antenna in living cockroaches is sinusoidally stimulated by its spontaneous swing at frequencies higher than 10 Hz. However, when the antennal swing is interrupted by external obstacles, the change of angular velocity of antennal movement must be larger than that caused by spontaneous rates of swing. Since cockroaches touch such obstacles by swinging their antennae during searching behavior (Okada and Toh, 2000; Okada *et al*., 2002), large velocity changes included in higher-frequency stimuli used in the present study must constitute components of the stimulus during such forced changes of the antennal swing.

Only sinusoidal displacement was used in the present study, because this could be easily manipulated to include multiple stimulus factors such as position, velocity and accelerating velocity. If the flagellum is moved very slowly at a frequency of say 0.5 Hz, the positional factor must be predominant. If, on the other hand, the flagellum is moved at

medium and high frequencies, such as at 50–150 Hz, the velocity and accelerating velocity must be more effective factors.

How receptor neurons are mechanically stimulated may be deduced from their discharge patterns to sinusoidal stimulation. To lower frequency stimuli at 0.5 Hz, 80% of receptor neurons examined did not show phase-dependent responses. This suggests that the receptor cells are less sensitive to the position of the flagellum, because a positional factor would be effective at such slow movements of the flagellum. Moreover, the remaining 20% of cells that showed phase-locked responses discharged spikes at a single phase of the stimulus cycle. The antenna must be at the same position twice during each stimulus cycle (e.g., 45° and 135° as well as 0° and 180°) except for the dorsal and ventral turning position (90° and 270°). However, few neurons discharged spikes at two such phases of the stimulus cycle.

The generation of tightly phase-locked spikes suggests that mechanical displacement for every stimulus cycle ought to be reliably transmitted to the receptor neuron. Such tight coupling between mechanical stimulation and spike generation is likely to occur if the sensory cilia are fixed to the structures surrounding their distal ends. Flagellar movement undoubtedly causes positional change of the cap structure against the proximal part of the sensilla housing the receptor neurons. This may be the case for one of the paired cilia in each sensillum, the one terminating with a swollen ending in the cap. The swollen ending fits so tightly to the lumen of the cap that it seems to be able to detect a minute displacement of the cap with respect to the receptor cell body. On the other hand, this form of tight coupling does not seem to be a probable explanation for the other of the paired cilia, because its slender ending does not appear to be trapped by the cap.

Another possible interpretation for the tight coupling between stimulation and spike generation is that the sensory cilium may behave like an indicator needle of the seismometer. Since the attachment cell is rich in microtubules and the scolopale cell possesses scolopale rods, the structure around the lumen housing the sensory cilia may be predicted to have some stiffness. The displacements of such a stiff surrounding structure may be more harmonized to stimulus displacement than the cilium, because the latter is floating in the narrow lumen. The cilium may be predicted to move, thus moving relative to the surrounding structure and the receptor dendrite. Such relative movement may, we suggest, mechanically stimulate the distal region of the dendrite, where the sensory cilium inserts.

It is not known how the sensory cilium is structurally transformed by mechanical stimulation to generate electrical responses. Most receptor neurons examined discharge spikes at a specific phase of the stimulus cycle, and the phase does not shift much over a wide range of stimulus frequencies. Constancy in the phase at which the spike is initiated suggests that the sensory cilium must be transformed almost in the same manner even if the duration of the stimulus cycle is changed considerably (i.e., 10 msec for stimulation with 100 Hz and 100 ms for that with 10 Hz). If some metabolic systems such as an internal second messenger system are involved in the early process, increases in stimulus frequency should cause a phase-shift in spike generation.

The idea proposed above is premised on the assumption that the sensory cilia are passively moved by sinusoidal stimulation. In the femoral chordotonal organ of the locust it has been proposed, however, that the sensory cilia might actively move in response to mechanical stimulation, and that ciliary movement might be detected at the distal part of the receptor neuron (Moran *et al.*, 1977). A train of precisely phase-locked spikes at a high stimulus frequency such as 100 Hz does not favor such an active role for the sensory cilium, however, because the beating rate of motile cilia is usually less than 30 Hz (Gibbons, 1981). An active role for receptor neurons in mechanoreception has also been suggested for the chordotonal sensilla of the tympanal organ in nocturnal moths (Coro and Kössl, 1998) as well as in Johnston's organ of the mosquito antenna (Göpfert and Robert, 2001), but a mechanism has not been demonstrated in either of these mechanoreceptors. Even if the receptor neurons actively participate in the process of mechanoreception examined in the present study, such a process may be auxiliary. To visualize such processes would require further study.

One of the basic questions posed by our study is whether the two sensory cilia in each sensillum differ in their response patterns. Insofar as the impaled receptor neurons were not morphologically identified, we cannot answer this question directly. However, some differences in the response patterns found in the present study may originate in the different morphology of the cilia. Since the phase-dependency of spike initiation in a given receptor neuron changed depending upon the stimulus frequency, the latter cannot apparently be related to the different morphologies of the two types of sensory cilia. It is possible, on the other hand, that the differences in the responses to stimulus frequency range between wide-frequency and high-frequency tuned neurons may be attributable to the different structures of the sensory cilia. In addition, differences in the optimal stimulus frequency for maximal response may be related to structural differences in the sensory cilia. Intracellular staining after recording will be required to address this question.

Most receptor neurons examined in the present study changed their discharge frequencies in response to both amplitude modulation and frequency modulation of sinusoidal stimulation. Given that changes of their impulse frequencies are modulated by at least two parameters, such receptor neurons can not individually tell their follower neurons in the brain how the antenna is stimulated. Therefore, the follower neurons must receive input from multiple receptor neurons to receive precise and unambiguous information on how the antenna is stimulated. However, a few receptor

neurons did change their spike frequencies in response to a modulation of stimulus frequency, but not of amplitude. These cells may thus code stimulus frequencies, but this still leaves the possibility that their responses may already have been saturated to stimulation at the lowest amplitude.

In the present study the responses of antennal chordotonal sensilla to sinusoidal displacement of the flagellum was examined. There still remain a number of questions concerning the mechanism of the primary process of mechanoreception. In order to answer such questions, other methods of stimulation, such as tension and ramp waves, will be required. Such approaches have been employed in our subsequent studies, and will be reported elsewhere (Ikeda *et al*., in preparation).

ACKNOWLEDGEMENTS

The authors express their thanks to Professor I. A. Meinertzhagen and Professor S. R. Shaw (Dalhousie University, Halifax, Canada) for invaluable comments throughout this work and for correction of the English. This work was supported in part by a Grant-in Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (11694091).

REFERENCES

Coro F, Kössl M (1998) Distortion-product of otoacoustic emissions from the tympanic organ in two noctuoid moths. J Comp Physiol A 183: 525–531

Göpfert MC, Robert D (2001) Active auditory mechanics in mosquitoes. Proc R Soc Lond B 268: 333–339

Field LH, Matheson T (1998) Chordotonal organs of insects. In "Advances in Insect Physiology Vol 27" ED by PD Evans, Academic Press, San Diego, pp 1–228

Gibbons IR (1981) Cilia and flagella of eukaryotes. J Cell Biol 91: 107–124

Heinzel H-G, Gewecke M (1979) Directional sensitivity of the antennal campaniform sensilla in locusts. Naturwissenchaften 66: 212–213

Moran DT, Varela FJ, Rowley III JC (1977) Evidence for active role of cilia in sensory transduction. Proc Natl Acad Sci USA 74: 793–797

Okada J, Toh Y (2000) The role of antennal hair plates in object-guided tactile orientation of the cockroach (*Periplaneta americana*). J Comp Physiol A186: 849–857

Okada J, Toh Y (2001) Peripheral representation of antennal orientation by the scapal hair plate of the cockroach *Periplaneta americana*. J Exp Biol 204: 4301–4309

Okada J, Kanamaru Y, Toh Y (2002) Mechanosensory control of antennal movement by the scapal hair plate in American cockroach. Zool Sci 19: 1201–1210

Toh Y (1977) Fine structure of antennal sense organs of male cockroach, *Periplaneta Americana*. J Ultrastruct Res 60: 373–394

Toh Y (1981) Fine structure of sense organs on the antennal pedicel and scape of the male cockroach, *Periplaneta americana*. J Ultrastruct Res 77: 119–132

Toh Y, Yokohari F (1985) Structure of the antennal chordotonal sensilla of the American cockroach. J Ultrastruct Res 90: 124–134

(Received August 11, 2003 / Accepted December 26, 2003)