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Mechanoreceptors involved in the hindwing-evoked escape behaviour in cricket, *Gryllus bimaculatus*

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Summary

Mechanoreceptors involved in the escape jumping evoked by hindwing stimulation have been investigated in the field cricket *Gryllus bimaculatus*. By partial ablation of the hindwing, we found that a mechanosensory system relevant to the escape behaviour was localized on specific veins of the hindwing tip. Scanning electron microscopy revealed three types of mechanoreceptive sensillae on the corresponding region. Based on their morphology, type I and type III sensillae were judged to be trichoid and chaetic sensillae, respectively. Type II sensillae were newly found in this study, having a twisted shaft with a socket-like structure at its base. They existed almost exclusively on the tip and middle regions of the hindwing. The conduction velocity of type II units was significantly smaller than that of type I units. One cycle of sinusoidal deflection of a single type II sensilla at frequencies in the range of 10–120 Hz caused the sensory unit to discharge a single or a few spikes that were not directly correlated with any specific direction of hair movement nor specific deflection angle. The response probability decreased with the stimulus frequency to be less than 0.1 at 0.2 Hz. The results suggest that type II sensillae would serve as contact mechanoreceptors with a low-cut filter property to obtain general information on the presence of stimuli on the hindwing tip rather than specific information on their precise positioning or movement.

Key words: cricket, *Gryllus bimaculatus*, mechanosensory, hindwing, escape jumping, sensilla.

Introduction

Sudden or noxious stimulation often evokes escape behaviour in many animals. The occurrence of escape behaviour depends on the intensity of particular stimuli, whereas the motor pattern of escape behaviour varies according to the nature or modality of the stimulus. Thus, mechanosensory stimulation of different parts of the body elicits escape behaviour with different directionality (Wine, 1984; Camhi and Tom, 1978). Auditory and tactile stimuli evoke escape behaviour with different motor patterns in insects (Stumpner and von Helversen, 2001). The stimuli are detected by appropriate sensillae on the body surface or sensory organs inside the body (Stumpner and von Helversen, 2001). Stimuli of different nature or modality are detected by different sensory systems that activate different motor command systems (Krasne and Wine, 1987; Tauber and Camhi, 1995).

In the cricket *Gryllus bimaculatus*, it is well known that, as in the cockroach *Periplaneta americana* (Camhi, 1984; Plummer and Camhi, 1981), the air current stimulus applied to the cerci evokes escape behaviour that consists of running forward away from the stimulus source (Gras and Hörner, 1992; Tauber and Camhi, 1995). The mechanosensory system that is responsible for detecting the stimulus and transmitting the sensory information to the motor centre for escape running has been intensively studied (Boyan et al., 1989; Hustert, 1978, 1985; Hörner, 1992). On the other hand, mechanical stimulation of the hindwing elicits another type of escape behaviour in cricket, consisting of initial jumping and subsequent running to avoid the stimulus (Hiraguchi and Yamaguchi, 2000). Behavioural and electromyographic studies have revealed that the movement pattern of legs in the initial jump is different to that in the jump of the locust *Schistocerca gregaria* (Heitler and Burrows, 1977; Tauber and Camhi, 1995). Using three types of mechanical stimuli, i.e. bending, touching with a paint brush and pinching with fine forceps, Hiraguchi and Yamaguchi (2000) studied which stimulus was most effective in eliciting the escape jumping. Although bending and pinching were found to be equally effective in eliciting a simple response involving kicking or running, pinching was the most effective in eliciting escape jumping. The mechanosensory system responsible for detecting the pinch stimulus and transmitting the information to the central nervous system, however, remains unknown.

Many types of mechanosensory sensillae, including trichoid, campaniform and chaetic sensillae, on the cuticular surface of the insect wing have been reported (Elliott, 1983; Gettrup, 1966; Schäffner and Koch, 1987; Fudalewicz-Niemczyk and

Rosciszewska, 1972). It remains to be clarified which types of mechanosensory sensillae are present on the distal surface of hindwing.

In the present study, we investigated, by partial ablation of the vein system, which part of the hindwing was responsible for detecting the touch and pinch stimuli to elicit the escape behaviour. We used a scanning electron microscope to quantitatively examine how and what types of mechanosensory sensillae were distributed over the wing surface. By directly stimulating each of the sensillae, we studied the physiological characteristics of afferent activities. The results showed that a specific type of mechanoreceptive sensillae, having characteristic structure and responsiveness, was abundantly present on the tip region of the hindwing that was responsible for detecting the stimulus resulting in escape jumping and running.

Materials and methods

Experimental animals

We used adult field crickets (*Gryllus bimaculatus* de Geer) that were 1–7 days after the imaginal moult. Electrophysiological studies were carried out with crickets that were within 24 hour after the imaginal moult. Animals were taken from a breeding colony in our laboratory held at 26–28°C under a 12 h:12 h light:dark cycle. Both sexes were used in this study. There were no noticeable differences between sexes in the results. Animals were anaesthetized with CO_2 before we made preparations. Thirty-three animals were used for morphological study and 56 animals were used for physiological study.

Morphology of the hindwing

The structural characteristics of the hindwing were examined under a dissecting microscope (SZH-131, Olympus, Tokyo, Japan). Fine details were compared with photographs of the hindwing taken with a microscopic camera (PM-20, Olympus). For scanning electron microscopy, the hindwing was isolated from the rest of the body. The specimen was then fixed in 100% ethanol, critical point freeze-dried in a vacuum evaporator, mounted on a peg and coated with gold–palladium. A scanning electron microscope (JSM-T300, JEOL, Tokyo, Japan) was used to compare the results of freeze-dried and naturally dried specimens. Veins and other parts of the hindwing were named according to Fudalewicz-Niemczyk and Rosciszewska (1972) and Brodsky (1994). In this study, the veins were numbered successively from the most anterior vein (Fig. 1C).

Behavioural experiment

In order to find out which part of the hindwing was responsible for receiving the effective stimulus for eliciting escape behaviour, selective ablation experiments were conducted. Experimental groups included animals with their forewings removed, those with the vannus of hindwing removed, those with the vannus and veins (#4, #5, #6 and #9) removed, and those with the veins (#2, #3, #7, #8 and #10) removed by cutting with scissors. The proximal half of the hindwing was left intact. The pinching stimulus was applied to the tip of the hindwing as described elsewhere (Hiraguchi and Yamaguchi, 2000). Each animal was stimulated five times. The rate of occurrence was obtained for each animal by dividing the number of responses by the number of stimulations.

Electrophysiological recording from the wing nerve

The hindwing was isolated from the rest of the body. The cut-end was protected against desiccation with petroleum jelly. For recording the type II unit activity, the cuticle on the dorsal side was removed at the branching point of veins #7 and #8 (Fig. 1C) to expose a branch of the wing nerve. A pair of hook electrodes was placed on the branch in the vein #7 or #8 and covered with petroleum jelly under a dissecting microscope. The electrodes were connected to a differential amplifier (MEG-2100, Nihon-Kohden, Tokyo, Japan) whose output was fed to an analogue oscilloscope (Tektronix 5100, Beaverton, USA) and stored on magnetic tapes using a DAT recorder (DTR-1801, Biologic, Claix, France; frequency range DC –20 kHz). In later analyses, the recorded signal was replayed and fed to PowerLab/8RSP (ADInstruments, Tokyo, Japan), which was controlled by Chart version 4.0 running on a PowerMacintosh 7300 personal computer. For measuring the conduction velocity of sensory units, the wing nerve between the hindwing and the metathoracic ganglion was exposed and isolated together with the wing. Two pairs of hook electrodes were placed along the nerve, separated from each other by approximately 2 mm. For unknown reasons, the physiological condition of the nerve rapidly deteriorated after exposure to saline. Thus, reliable recording was possible for less than 30 min.

In order to examine the activity of wing proprioceptors, we made a head–thorax preparation with the hindwing intact on one side (Hiraguchi and Yamaguchi, 2000). An extracellular suction electrode was placed on the N2D2 of the metathoracic ganglion. This nerve contains only those axons from the wing proprioceptors (Kutsch and Huber, 1989). The pinching stimulus that was made manually with fine forceps was monitored by measuring the electrical resistance between the forceps and the insect body.

Mechanical stimulation

The single mechanosensory sensilla was directly stimulated with a fine tungsten stylus (50 μm in diameter) sharpened by electrolysis. The stylus was attached to a loud speaker (8 Ω impedance, 0.5 W) that was driven by the output of a hand-made amplifier with a current booster circuit. A single cycle of sinusoidal signal was produced by a function generator (3312A, Hewlett-Packard, Palo Alto, USA) and fed into the amplifier. The stimulus was started at the lower reversal point of the sine wave in order to avoid sudden movement at the onset of stimulus. The position of the stylus relative to the sensilla was fine-tuned by DC offset of the

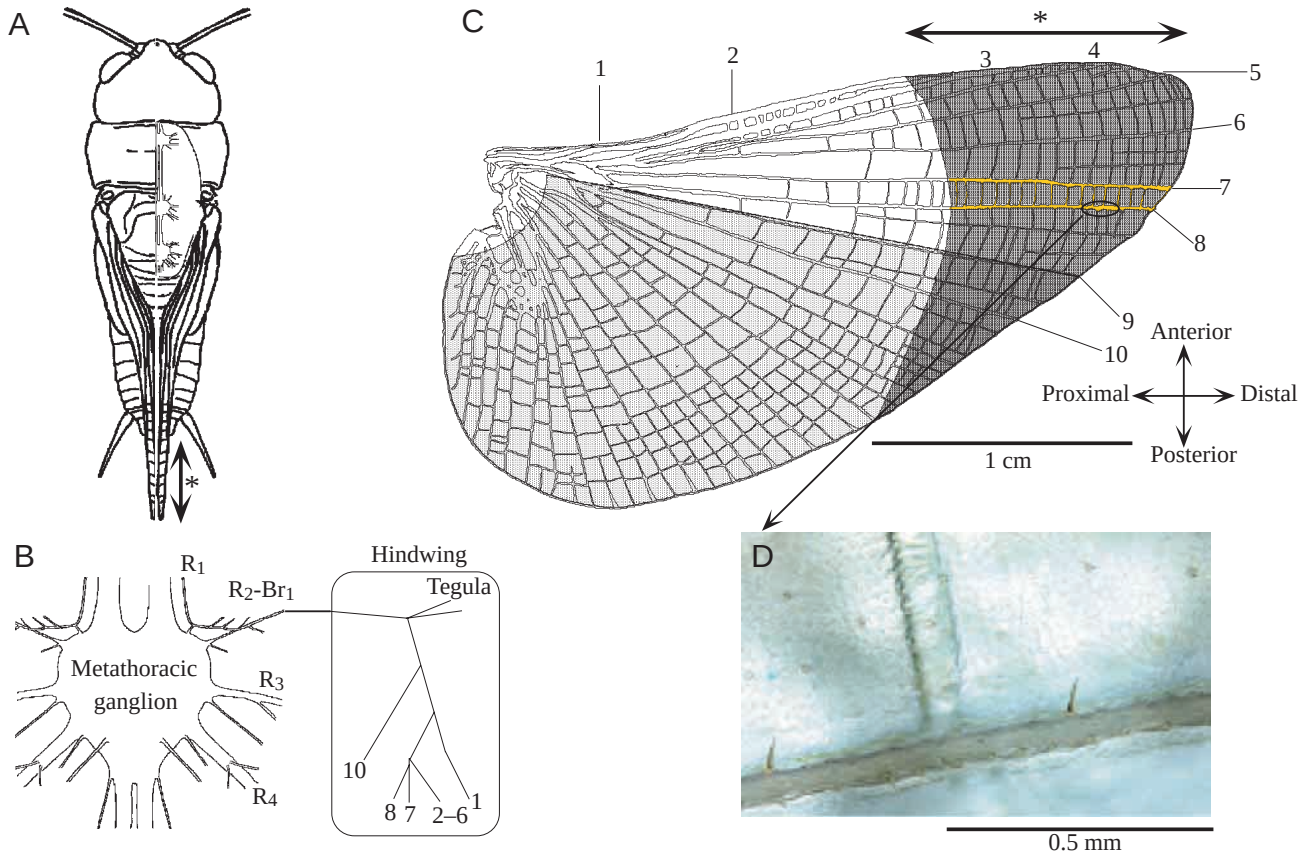

Fig. 1. Morphology of the hindwing and vein diversion. (A) Dorsal view of the hindwing. The forewing was removed to expose the whole hindwing in its folded position. (B) Metathoracic ganglion and schematic diagram of the wing nerve branching pattern, showing nerve roots (R) and branched nerves (Br). (C) Schematic drawing of the hindwing extended. Veins are numbered successively from the most anterior (#1) to the most proximal (#10). The dark grey region corresponds to the portion indicated with an asterisk in A. The proximal part of the hindwing is covered by the forewing to the level of the 17th or 18th cell from the most distal cell (mean ± S.E.M. 17.7±0.2; *N*=5). The light grey region indicates the vannus region. The remaining part is called the remigium. (D) Optical microscopic view of the encircled part in C.

function generator. Movement of the stylus was monitored not by the driving signal but by a phototransistor coupled with a light-emitting diode between which the stylus was positioned. The stylus could follow up to 120 Hz. The stylus was placed just in contact with the sensilla under a microscope (BX-60, Olympus) with an objective (×20) having 7.5 mm working distance. Depending on the stylus position, the sensilla was lifted or further lowered from its lying position during the first half of the single sinusoidal cycle and returned to the original position during the second half. The return of the sensilla to its original position was due to its elasticity. The shape of the stylus was adjusted for each hair and for stimulus direction.

In the experiment to measure the conduction velocity of sensory units associated with type I, II and campaniform sensillae, we used different types of stylus suited for stimulation of each type of sensillae and adopted pulse function with the duration of 100 ms, instead of a sinusoidal cycle, as the stimulus profile for stylus movement to activate the sensillae as securely as possible. The pulse interval was adjusted in each preparation according to the frequency of background spike discharge in order to unambiguously discriminate the elicited spikes from the spontaneous ones.

Results

Hindwing morphology

The hindwings of the cricket are usually folded to cover its body on the dorsal side during resting as well as in action (Fig. 1A). The forewing further covers the basal and middle parts of the hindwing, but its distal part is exposed to the surroundings, protruding more posteriorly than the anus (shown by an asterisk in Fig. 1A). Nerves from the metathoracic ganglion innervate the hindwing *via* the first branch of the second nerve root (Fig. 1B). This nerve branch bifurcates in the hindwing hinge. One branch innervates the tegula, corresponding to $n1C_1$ in locust, while the other branch extends into the wing, corresponding to $n1C_2$ in locust. The latter branches into three fine roots, one of which innervates veins #7 and #8.

The dorsal view of the hindwing is illustrated in Fig. 1C,D. In this study, the veins were numbered successively from the

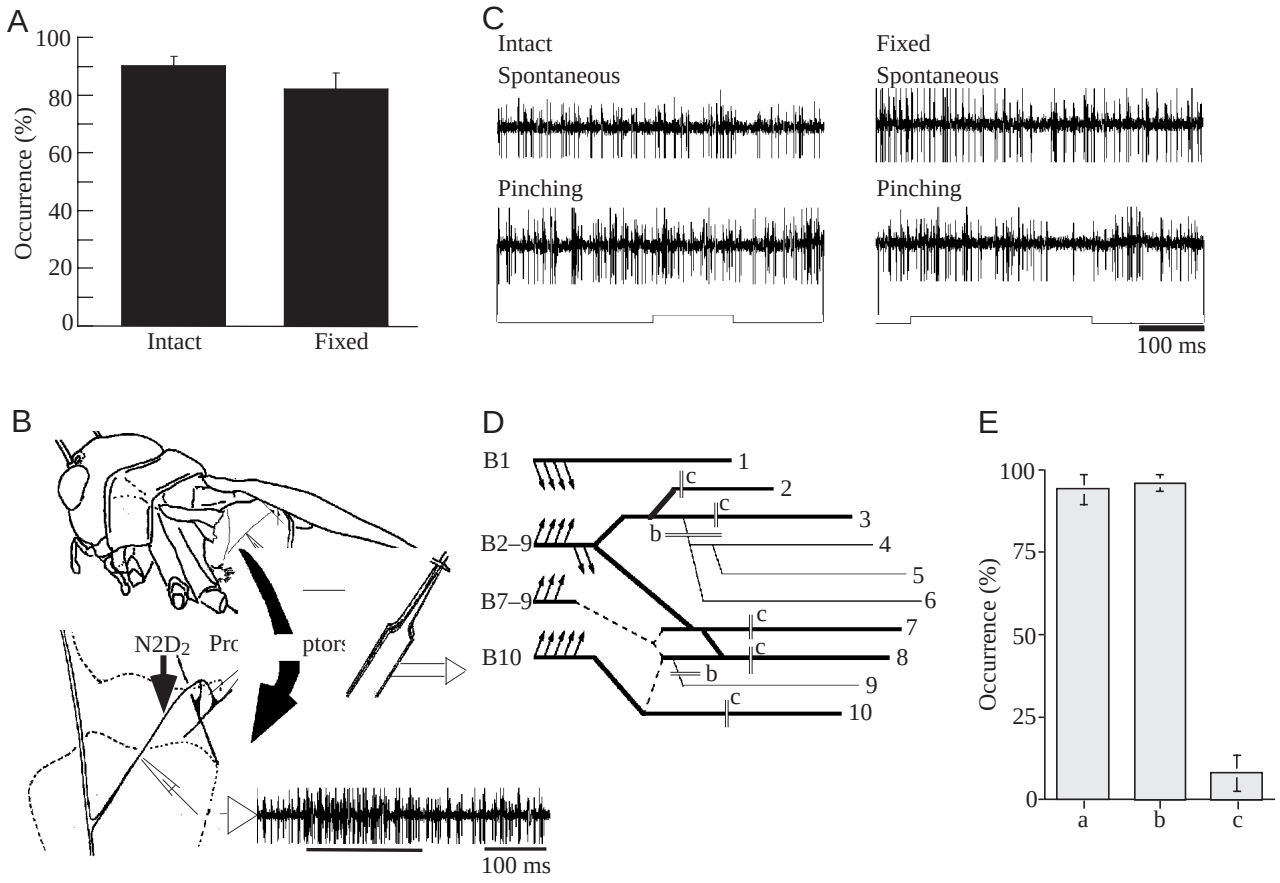

Fig. 2. Functional region of the hindwing for detecting mechanical stimuli that elicit escape jumping. (A) Comparison of the occurrence probability of escape response to the pinching stimuli applied to the hindwing tip. There was no significant difference between intact animals and those animals with the hindwing immobilized (fixed). (B) Experimental setup for recording neural activities from the nerve branch supplying hindwing proprioceptors. Adapted from Kutsch and Huber (1989). A sample record is also shown. (C) Activities of proprioceptors recorded extracellularly. Left and right panels show activities in intact and fixed conditions, respectively. In each panel, the upper trace shows spontaneous activities, whereas the lower trace shows activities during pinching stimulation monitored by the bottom trace. (D) Schematic drawing of the branching pattern of veins #1–#10. Arrows indicate type I sensillae. The line widths indicate the relative thickness of each vein. The broken lines show thin cuticular layer parts in veins. The double lines show the cutting point for the experiment shown in E and lower case letters show experimental conditions. (E) Comparison of occurrence probability of escape response to the pinching stimuli in animals with the hindwing partially removed. A, the vannus removed; b, the vannus and veins #4, #5, #6 and #9 removed; c, veins #2, #3, #7, #8 and #10 removed.

most anterior vein (Fig. 1C). Six of the veins (#1, #2, #3, #7, #8 and #10) were thick, having a rather massive, brownish cuticular layer. The thin cuticular membrane between veins was partitioned into several cells by cross veins (Fig. 1D). Between veins #7 and #8, the membrane was made of thick cuticle. The cells were numbered successively from the most distal cell. The tip of the forewing that overlapped with the hindwing was located at the level of the 17th or 18th cell (17.7±0.2; mean ± S.E.M., *N*=5). Proximal cells were entirely covered by the forewing. The costa, i.e. vein #1, was isolated from the rest of the hindwing veins at its basal part, being shorter than the other veins. Veins #2–#9 branched out from a few veins at the basal part of the hindwing and extended to the distal part of the hindwing. Vein #10 emerged from another vein at the basal part. They were placed next to each other when the hindwing was folded. In the middle part of the folded hindwing, six veins (#2, #3, #4, #7, #8 and #10) were placed on the most dorsal side, and only two of them (#7 and #8) were on the most dorsal side in the distal part of the folded hindwing. Veins #7 and #8, together with the membrane between them, were directly exposed to the external world.

Role of proprioceptors in evoking escape behaviour

It has been reported that many kinds of proprioceptors are located at the base of the hindwing and in the thorax (Altman and Tyrer, 1974; Gettrup, 1966) to detect the position and movement of the hindwing. In order to test the possibility that they trigger the escape behaviour in response to mechanical stimulation of the hindwing, we immobilized the proximal part of the hindwing (approximately two-thirds of the whole wing) by fixing it to the abdominal tergum with a piece of cover glass using wax. The glass was used to prevent, by its own weight,

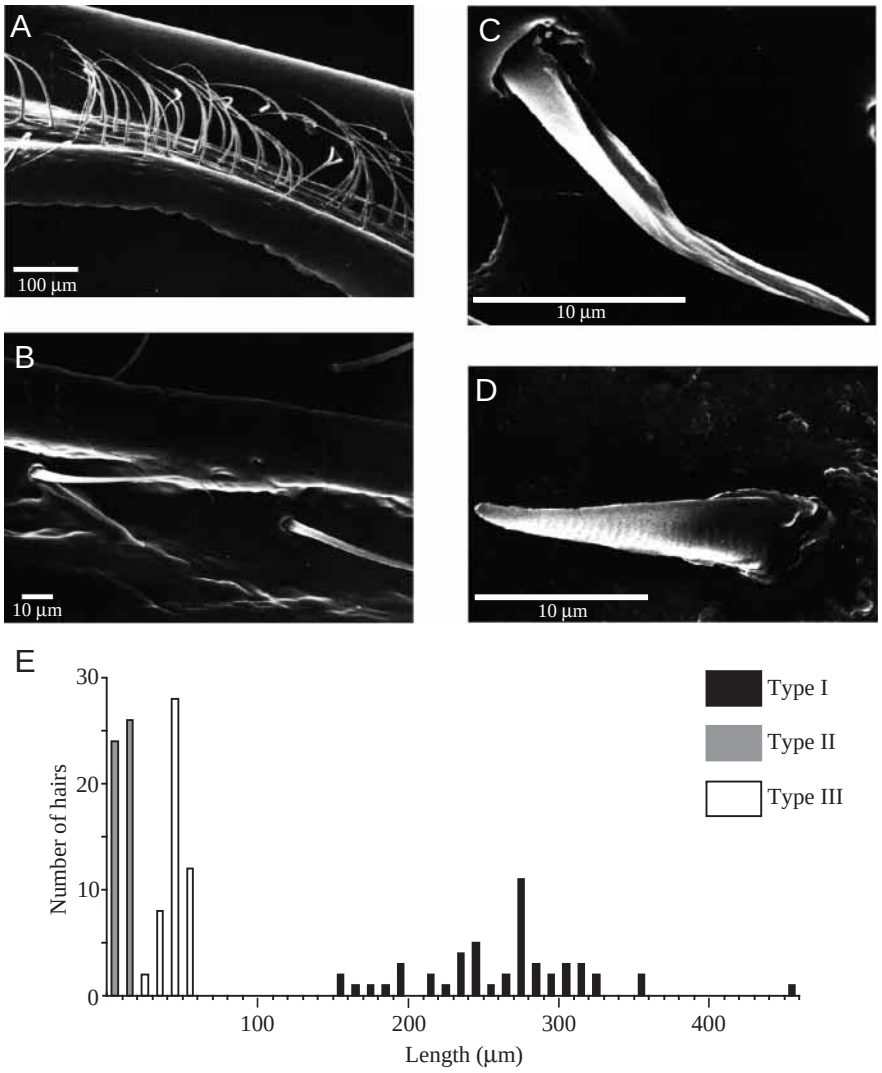

Fig. 3. Structure of mechanosensory hairs on the hindwing surface examined by a scanning electron microscope. (A,B) Type I sensillae. (C,D) Type III sensillae. (E) Histogram showing the length distribution of sensillae on the hindwing.

transmission of the wing movement caused by pinching stimulation to thoracic proprioceptors. The distal half of the forewing was removed. Each of 10 experimental animals was stimulated five times, with an interval between stimulations of at least 3 min, by single pinching applied manually to the hindwing tip. The rate of response was obtained for each animal by dividing the number of responses by the number of stimulations. The rate of occurrence of escape behaviour in response to pinching stimulus in the experimental animals (82.0±6.5%) was not statistically different (P>0.05; Student's two-sided unpaired t-test; Fig. 2A) from that in intact animals (90.0±3.3%). The result indicated that the sense organs responsible for detecting the mechanical stimulus applied to the hindwing to elicit escape behaviour were present not at the base but on the surface of the hindwing. We also confirmed physiologically that pinching stimuli evoked no significant response of the proprioceptors in either the intact or fixed condition (Fig. 2B,C; N=6).

The branching pattern of veins in the hindwing is illustrated in Fig. 2D. Six thick veins were located in the remigium region. Of these, veins #7 and #8 were the longest. In animals with their forewings ablated, removing a specific region of the hindwing significantly affected the occurrence of escape behaviour elicited by the pinching stimuli applied to the tip of the hindwing (Fig. 2E). When the vannus of hindwing was totally removed (group 'a' in Fig. 2E), the rate of occurrence was 94.0±4.2% (N=10 animals). When the vannus and veins #4, #5, #6 and #9 were removed (group 'b'), the occurrence rate was 96.0±2.6% (N=10 animals). When veins #2, #3, #7, #8 and #10 were removed (group 'c'), however, the average rate of occurrence was 8.0±5.3% (N=10 animals). The difference in the rate of occurrence among the three groups was statistically significant (P<0.001; single classification ANOVA). Planned comparisons among pairs of means (Sokal and Rohlf, 1995) revealed that the occurrence rate for group 'c' was significantly lower than the rate for groups 'a' and 'b' (P<0.001 for both). These results suggested that the mechanosensory organs for detecting the stimuli to elicit escape behaviour were located on veins #2, #3, #7, #8 and #10.

Sensillae on the hindwing surface

Scanning electron microscopy has revealed that there are several types of hair-like structure on the cuticular surface of the hindwing. In the remigium region of the hindwing, we identified three types of sensory hair structure that existed on some of the veins and cross veins and on the specific part of membranous cells surrounded by them. The observation that these hairs rested on a socket-like structure suggested that they were all mechanosensory sensillae. In this study, those hairs with a smooth and thread-like shaft were classified as type I sensillae. They were all longer than 100 μm (mean ± S.E.M., 264.0±11.0 μm, N=50 from six wings; Fig. 3A,B) and their morphology resembled that of sensillae on the cerci of the cricket and locust (Boyan et al., 1989; Gnatzy and Hustert, 1989; Murphey, 1985) as well as on the body and appendage surface of other arthropods (Gronenberg and Tautz, 1994). Those hairs with a stout and bristle-like shaft were designated as type III sensillae (Fig. 3C,D). Being short in length (45.5±1.0 μm, N=50 from five wings), they appeared to correspond to the bristle sensillae reported in the cricket (Boyan et al., 1989; Hamon and Guillet, 1996; Murphey,

1985). The length-distribution histogram (Fig. 3E) showed that type I sensillae were discontinuously longer than other sensillae, including type III sensillae.

In the present study, for the first time, we found hairs with a shaft that was characteristically twisted and typically resting in a deflected position, paralleling or making contact with the cuticular surface (Fig. 4). They were relatively small at low magnification (Fig. 4A), but observation under higher magnification revealed their twisted structure (Fig. 4B). Statistical association between the twisted structure and the hair length was demonstrated to be significant using a χ^2 test (P<0.001), indicating that the two populations of hairs, either having or lacking the twisted structure, were different in their hair length. Although the hair shown in Fig. 4B looks like it is standing straight up from the cuticular surface, it was in reality deflected towards the surface, as shown in Fig. 4C. No sensory hair structures so far reported in other mechanosensory systems appear to correspond to the type II sensillae. They were significantly shorter (10.4±0.2 μm, N=50 from five wings) than both type I and type III sensillae (P<0.01 for both; Student's two-sided unpaired t-test). As campaniform sensillae are well known for reception of cuticular distortion (Schäffner and Koch, 1987), which is likely to be caused by pinch stimulation, we looked for this type of sensilla carefully in this study. However, no evidence was found that they were present on the hindwing tip.

We have counted under a microscope the number of mechanosensory hairs on the cell surface between, and including, veins #7 and #8 using five wings from five animals. It was found that type II sensillae were most abundant on the surface of the middle to distal region, i.e. the sixth and seventh cells from the most distal cell, decreasing in number both in the proximal and distal directions: there were approximately 22 type II sensillae on the seventh cell compared with two sensillae on the most distal cell and on a proximal (i.e. 20th) cell (Fig. 5A). They were mostly confined to the distal part of each cell on its dorsal side (Fig. 8). No other veins apart from #7 and #8 were found to carry type II sensillae. These sensillae were also distributed on the surface of veins #7 and #8 uniformly over their length on the hindwing (Fig. 5B). By contrast, type I sensillae were found to exist only on the proximal region. Type III sensillae were only scarcely present on the surface of all cells, with a mean number of 1.9±0.2 sensillae on each cell (Fig. 5C).

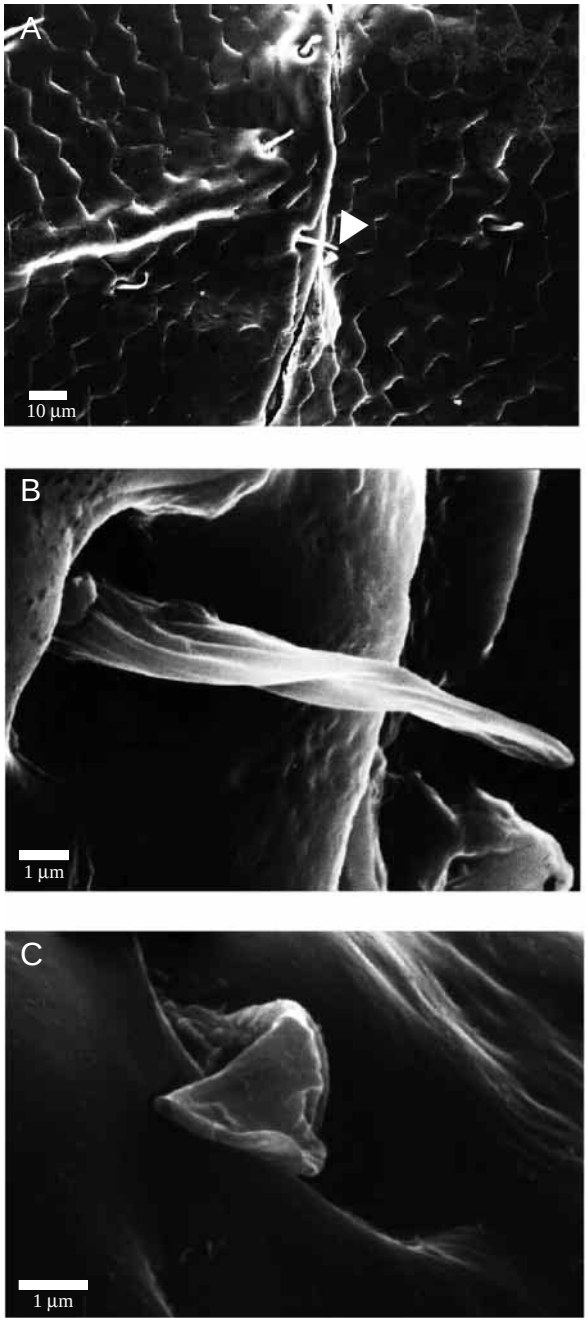

Fig. 4. Structure of type II sensillae. (A) An arrowhead indicates the sensilla. The sensillae exist near the cross vein, which is swollen in the distal part of each cell. (B) The twisted hair shaft. Many grooves were seen on the surface. Distal is to the right, anterior to the top (A,B). (C) The hair shaft deflected towards the cuticular surface. Viewed from the distal direction. No pore was found on the tip of the shaft.

Conduction velocity

In order to measure the conduction velocity of sensory nerves associated with the type II sensillae, we stimulated the sensillae and made extracellular recordings from the nerve axon using two pairs of hook electrodes. For comparison, we also measured the conduction velocity of nerves associated with type I and campaniform sensillae. In the experiments illustrated in Fig. 6A–C, each type of sensillae was selectively stimulated and their nerve activity was recorded at two different sites along the wing nerve (Fig. 6D) using two pairs of hook electrodes separated from each other by approximately 2 mm. As the campaniform sensillae were not found on the hindwing tip region, we stimulated those found on the proximal part of the hindwing. The location of stimulated sensillae is shown schematically in Fig. 6D. For recording type I unit activity, the wing nerve was severed distally to the site of stimulation in order to make the unit activity discernible

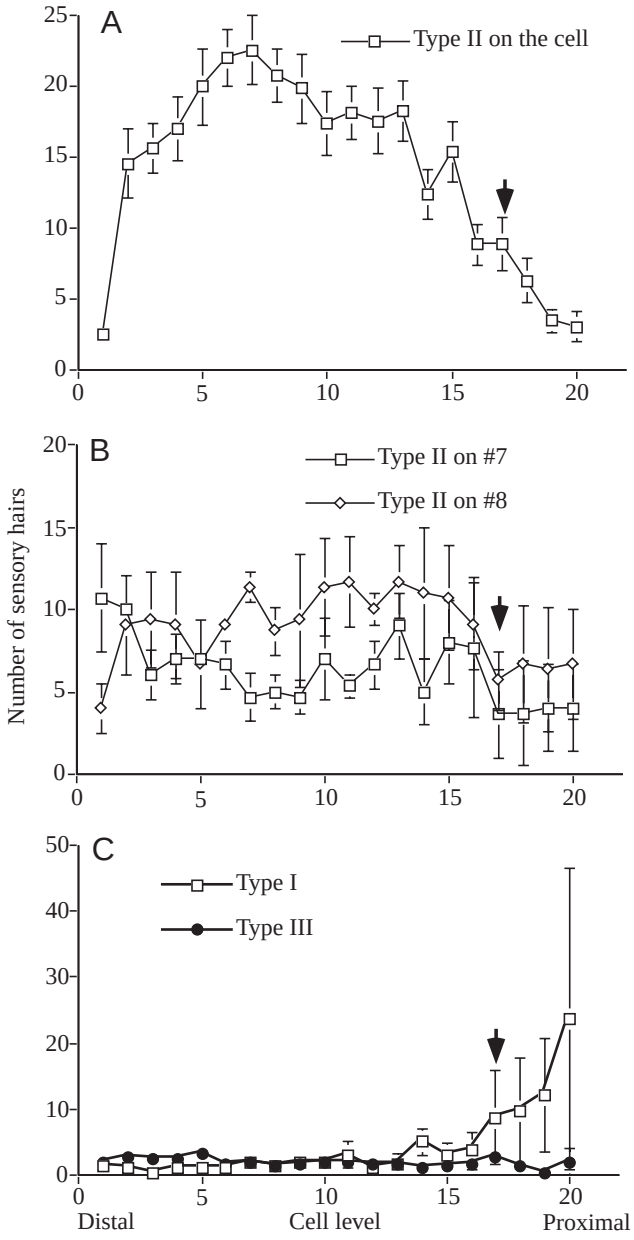

Fig. 5. Number of sensory hairs on the dorso-distal part of the hindwing. Means ± S.E.M. are shown. (A) Type II hairs on each cell between veins #7 and #8. Cells are numbered successively from the most distal cell, #20 being the most proximal cell. (B) Type II hairs on veins #7 and #8. (C) Type I and type III hairs on veins #7 and #8. An arrowhead shows the cell to which the hindwing was covered by the distal part of the forewing.

from reduced spontaneous spike discharges (Fig. 6A). In other recordings, the whole hindwing nerves remained intact: the type II and campaniform unit activities were observed among many spontaneous spikes but were unambiguously discernible as they were locked to the stimulus onset (Fig. 6B) or onset and offset (Fig. 6C). Since we simultaneously stimulated several sensillae of the same type for reliable recording of the unit activity, several units were observed to be activated in a single stimulation (Fig. 6A–C). We selected one or two discernible units and measured their conduction velocity by dividing the distance between electrodes by the delay time.

The conduction velocity of type I, type II and campaniform units is summarised in Fig. 6E. The conduction velocity of the type I unit (2.4±0.5 m s^{-1}; N=10 units from eight animals) was found to be as fast as that of the campaniform unit (2.3±0.1 m s^{-1}; N=5 units from three animals; P>0.05, Student's two-sided t-test), whereas the conduction velocity of the type II unit (1.4±0.1 m s^{-1}; N=6 units from six animals) was significantly slower than those of the other types of units (P<0.05). In accordance with its high conduction velocity, the type I unit showed significantly large spike amplitude (465.1±20.0 μV; P<0.05) compared with that of the type II unit (177.6±7.9 μV). The spike amplitude of the campaniform unit (334.9±8.4 μV) was not statistically different from that of the type I unit (P>0.05). The difference between the spike amplitudes of the type II and campaniform sensillae was statistically significant (P<0.05).

Afferent responses to stimulation of a single type II sensilla

For studying the response characteristics of type II sensillae, we adopted sinusoidal stimulation, instead of the rectangular stimulation adopted in the preceding experiment (Fig. 6). A single type II sensilla was deflected sinusoidally, using one cycle starting from the minimum point at varying frequency (0.1–120 Hz). Each stimulation was separated by an interval of ≥60 s. When the sensilla was lifted from and returned to its initial lying position (Fig. 7A), almost no spike discharge was observed at low frequencies (<1 Hz). At higher frequencies, the sensory nerve connected with the sensilla usually responded with a single or a few spikes. The spike response was always phasic: sustained spike discharge was never observed in this study, although the sensilla remained deflected for a while during stimulation. When the cuticular surface in the vicinity of the sensilla was directly stimulated, no response was recorded (Fig. 7A). Since the recording electrode was placed several mm away from the sensilla, the relative timing of spike discharge to the stimulus monitor varied depending on the stimulus frequency. Even with the same stimulus frequency, the timing of spike discharge showed fluctuation, as exemplified in the responses to 10 Hz stimulation in Fig. 7A. The fluctuation was also observed when the sensilla was lifted from and returned to the original position (Fig. 7B). The timing of spike discharge fluctuated over a range of >10 ms in 10 Hz stimulation. These observations suggested that activation of the sensory unit associated with the type II hair would not be strictly related to its deflection angle or direction. It thus appeared that a single type II sensilla would not encode detailed information on the stimulus; instead, it would carry general information on whether the stimulus to the hindwing tip is present or not when the stimulus is fast enough (>1 Hz).

It was also noted that the response of a single type II unit to repeated stimulation was of probabilistic nature (Fig. 7C). When the stimulus of the same frequency and amplitude was repeated 10 times, the probability of spike discharge increased

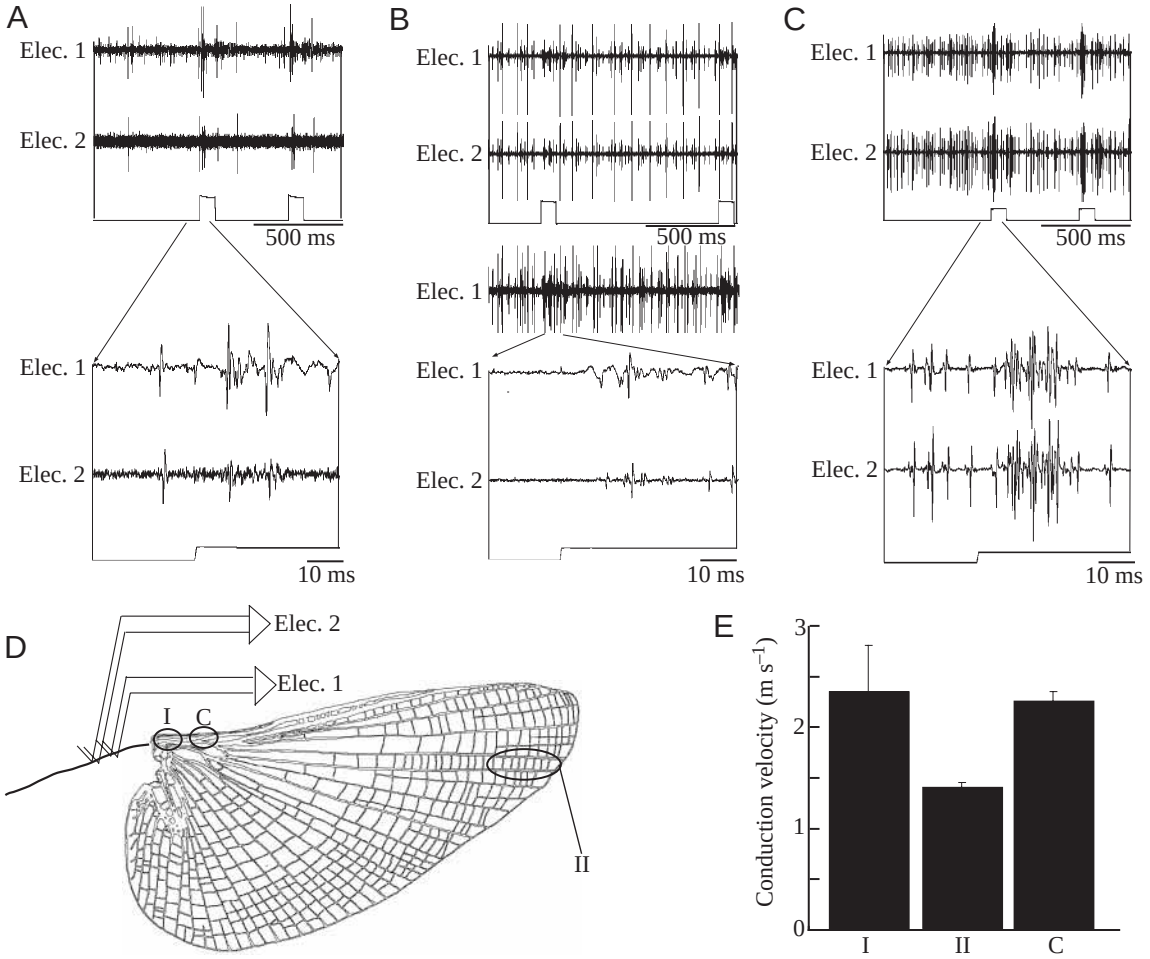

Fig. 6. Conduction velocity of the hindwing mechanosensory signals. Two pairs of hook electrodes were placed on the wing nerve separated by 1.8 mm from each other. (A) Responses to stimulation of type I sensillae on the proximal part of the hindwing. To eliminate spontaneous spike activity of other units, the distal portion of the wing nerve was crushed. The upper (elec. 1) and lower (elec. 2) records were obtained by the distal and proximal electrode, respectively. The bottom trace monitored the stimulus. The lower panel is a partial expansion of the upper panel. (B) Responses to stimulation of type II sensillae on the distal part of the hindwing. The trace between the upper and lower panels is the high-gain reproduction of the record shown in the upper trace (elec. 1). (C) Responses to stimulation of campaniform sensillae on the proximal part. (D) Experimental setup and location of each type of sensillae stimulated in the experiment. (E) Conduction velocity of the sensory units associated with each type of sensillae. I, II and C indicate type I, II and campaniform sensillae, respectively.

with the stimulus frequency up to about 0.7±0.1 at 10 Hz (N=100 in 10 animals). At frequencies higher than 10 Hz, the probability remained unchanged except at 50 Hz. The finding that the maximal probability of spike discharge in response to stimulation was approximately 0.7 indicated that a single type II sensilla by itself would not be able to detect the stimulus with adequate precision for eliciting escape jumping. This disadvantage appears to be compensated for by high-density distribution of type II sensillae on the hindwing tip region (see Discussion).

We also examined the response characteristics of 12 type II sensillae in the same preparation (Fig. 8). Most of them (11/12) responded to more than three out of five stimulation trials with a single or a few spikes. None of the examined units in this experiment responded with spike discharge to every trial of stimulation. One hair, shown in the top-left corner in Fig. 8, never caused spike discharge upon stimulation. This failure appeared to be due to inadvertent damage to the nerve or to unfavourable axon location within the nerve for the recording electrode. The latent period from the stimulus onset to the first spike discharge in Fig. 8 ranged from 26.4 ms to 28.9 ms (mean ± S.E.M., 27.8±0.2 ms). This variability was partly due to unintentional differences in the positioning of the stylus for each hair but also appeared to reflect the unstable timing of spike discharge in response to deflection of the same hair (Fig. 7). Although a single type II unit was not reliably responsive to hair deflection even within the preferred frequency range, we concluded that the animal would be able to respond with escape jumping to the stimulus applied to the hindwing tip by monitoring the spike activity of a population of type II sensillae that are present almost exclusively and close together on the exposed surface of the hindwing.

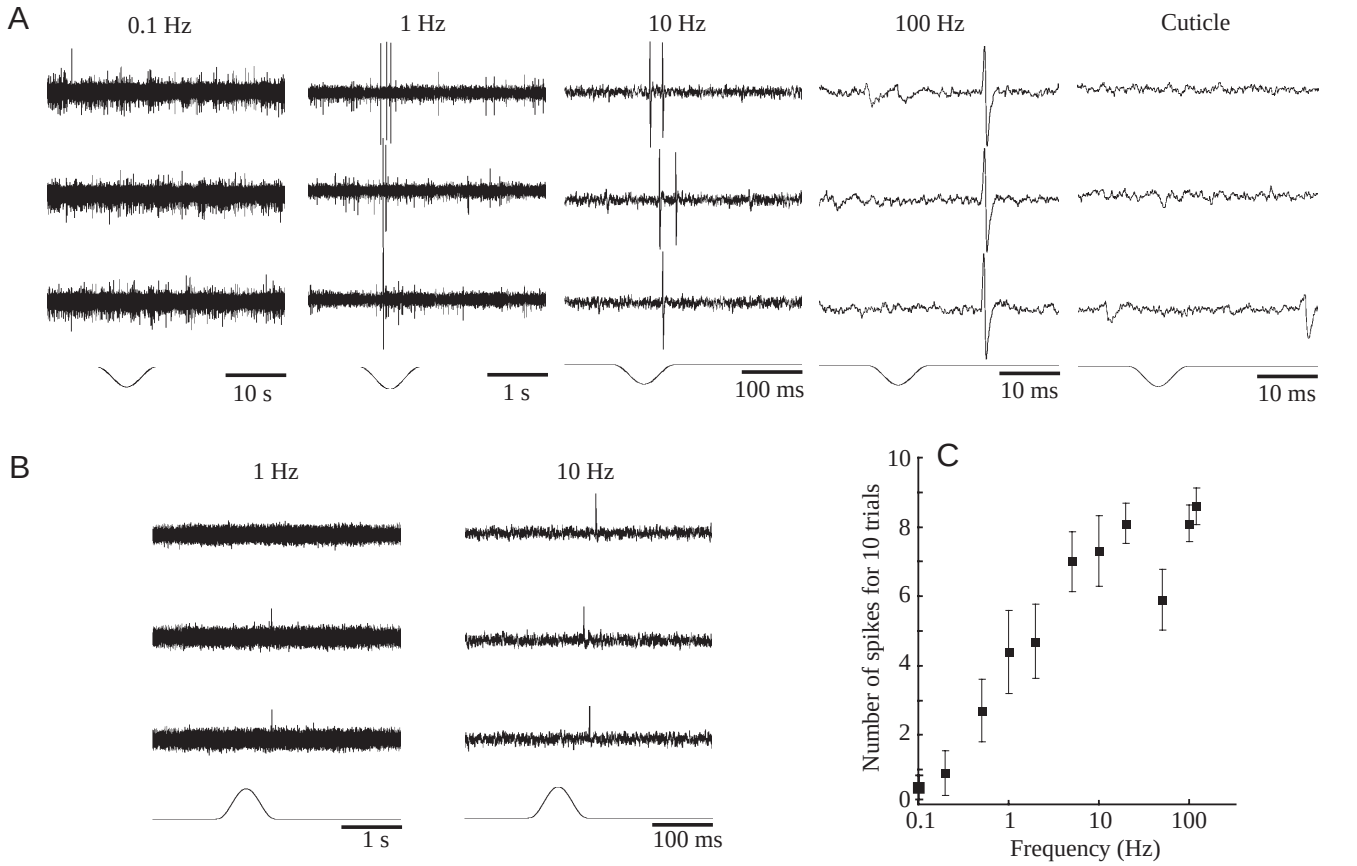

Fig. 7. Afferent responses to stimulation of a single type II sensilla. (A) The hair shaft was deflected toward the cuticular surface and returned to its original position with a tungsten stylus that was moved by a single cycle of sinusoidal function at varying frequencies (0.1 Hz, 1 Hz, 10 Hz and 100 Hz). Three representative records are shown for each frequency. The bottom trace monitors the stimulus. The rightmost panel shows the record when the cuticular surface in the vicinity of the deflected sensilla was stimulated to demonstrate that the observed response to 1–100 Hz stimulation was directly caused by shaft deflection. (B) The hair shaft was lifted up from the cuticular surface and returned to its original position. The polarity of the stimulus monitor (bottom trace) is reversed accordingly. Responses to stimulation at 1 Hz and 10 Hz are shown. (C) Number of elicited spikes for 10 stimulation trials plotted against stimulus frequency. The chart is based on the type II unit responses to lift-up stimulation exemplified in B.

Discussion

The present results demonstrate that the escape jumping of the cricket *Gryllus bimaculatus* in response to mechanical stimulation of the hindwing tip (Hiraguchi and Yamaguchi, 2000) is elicited by a novel class of mechanoreceptive sensillae that are characteristically distributed over the cuticular surface of the hindwing tip. In the well-known escape behaviour of crickets and cockroaches in response to air puffing applied to cerci, the stimulus is detected by filiform sensillae that are mechanically adapted for detecting specific aspects of the air current (Kanou et al., 1988; Kanou and Shimozawa, 1984; Shimozawa and Kanou, 1984a,b; Murphey, 1985). The sensillae newly found in this study have a characteristic structure that clearly distinguishes them from filiform or trichoid sensillae. In the following sections, we discuss the structural and functional characteristics of the novel sensillae in relation to escape jumping.

Hindwing as the sensory organ

There are many studies to date about the insect wing as the flight organ (Ellington, 1991; Brodsky, 1994). Studies on the sensory function of the wing have been mostly focused on mechanoreceptors related to flight control. Many types of receptors have been reported on or in the wings: filiform and campaniform sensillae on the basal part of the forewing (Fundalewicz-Niemczyk and Rosciszewska, 1972; Elliott et al., 1982) and stretch receptors attached to the forewing hinge (Schäffner and Koch, 1986; Gettrup, 1966). In flying insects, those sensory organs detecting the distortion of the wing in the proximal part of hindwings during flight have been studied in detail regarding their morphology and physiology (Yack and Fullard, 1993). Although Matheson (1997, 1998) reported that stimulation of hindwing tactile receptors elicited scratching movements of a hind leg in locusts, the sensory function of hindwings largely remains to be thoroughly examined.

The field cricket *Gryllus bimaculatus* has a relatively longer pair of hindwings than forewings (Fig. 1A,C), with a vein diversion pattern as simple as that of primitive species (Brodsky, 1994). It has many long and straight veins, and a lot of short, straight cross veins. Almost an entire portion of the

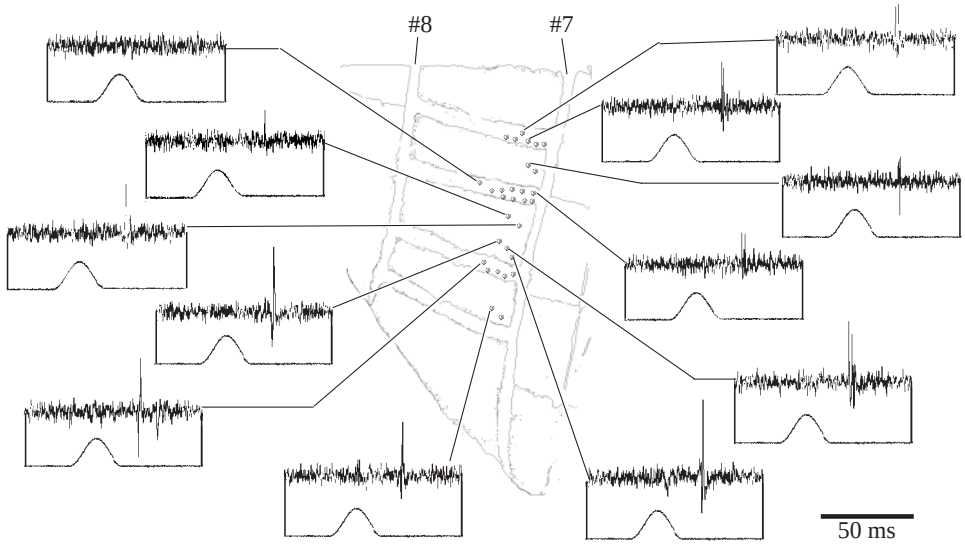

Fig. 8. Stimulation of type II sensory hairs on the distal part of cell series between veins #7 and #8 in the same preparation. Most units responded with one or a few spikes to a single stimulus. Distal is to the bottom, anterior to the right.

hindwing is covered by the forewing, only the distal part being exposed to the external environment in *Gryllus*. In the present study, we found that a new sensory system resided on this part of the hindwing. The results suggested that the hindwing would play an important and unique role in controlling behaviour, acting together with antennae and cerci.

Reception site of mechanical stimulation

We demonstrated by immobilization experiments (Fig. 2) that the mechanosensory stimulus applied to the hindwing tip to elicit escape jumping was received by exteroceptors on the hindwing rather than proprioceptors at its base or in the thorax. By immobilizing the hindwing and ablating the whole forewing, we found that the distal part of the hindwing would play an important role in detecting the mechanical stimuli. Partial ablation of the hindwing vein system further showed that the mechanosensory receptors responsible for receiving the escape-eliciting stimuli were mostly distributed on veins #7 and #8 (Fig. 2B). Examination of the cuticular surface of the hindwing using a scanning electron microscope revealed three morphological types of sensory hairs. Type I and type III hairs (Figs 3, 4) appeared to be the same as filiform (Gnatzy and Hustert, 1989; Murphey, 1985) and bristle sensillae (Boyan et al., 1989; Hamon and Guillet, 1996), respectively. Type II hairs, in contrast, appeared to be a novel type, as no known sensillae in insect correspond to these hairs (Mclver, 1985; Schwartzkopff, 1964). It should be noted here that, although we did not encounter other types of sensillae in the present study, this does not entirely exclude the possibility that, for example, campaniform sensillae or internal multipolar receptors might also be present. Further study is needed to test this possibility.

Quantitative observation has revealed that the type II sensillae were more abundant than the other two types of sensillae on the membranous cells between veins #7 and #8 in the distal to middle regions. Type I sensillae were found only in the proximal region of the hindwing on the veins #1–#9. Type III sensillae were very sparse throughout the membrane between veins #7 and #8 (Fig. 5). These findings suggest that type II sensillae are responsible for detecting the mechanosensory stimuli and transmitting the sensory information to the central nervous system.

Morphological characteristics of type II sensillae

Compared with the filiform sensillae on cerci, which have been reported to be involved in detection of wind stimuli in crickets, the type II sensillae on the hindwing are significantly shorter (10.4±0.2 μm; filiform sensillae, 158.0±6.8 μm). Characteristic to the type II sensillae was the twisted shaft (Fig. 4). Grooves on the shaft surface running in the axial direction clearly indicate the twisted structure. The whole shaft was most typically deflected at rest, paralleling or making contact with the cuticular surface. We think that these structural characteristics of type II sensillae are not artifacts but reflect their original morphology, as filiform sensillae, termed type I in this study, generally stood up vertically on the cuticle with straight external appearance in the same preparation. The type II sensillae (10.4±0.2 μm in shaft length) were found to be significantly shorter (P<0.01) than type I (264.0±11.0 μm) and type III (45.5±1.0 μm) sensillae.

Filiform sensillae on the cerci of *Gryllus bimaculatus* have been reported to range from approximately 30 μm to 1500 μm in length, thus having compatible length with type I sensillae on the hindwing tip (Fig. 3A–C). The cercal sensillae are receptive for air current stimuli (Dumpert and Gnatzy, 1977; Boyan et al., 1989): depending on the hair length, filiform hairs are thought to be specialised in detecting wind velocity or acceleration (Kanou and Shimozawa, 1984; Shimozawa and Kanou, 1984b). Having a short and crooked shaft, rather than the long and straight shaft of wind-sensitive filiform hairs, the type II sensillae (Fig. 4) are unlikely to be receptive for air current stimuli. The fact that in some cases the type II hair shaft was in contact with the cuticular surface further supported this possibility. In the course of this study, we actually observed

that wind stimulation applied to the hindwing tip elicited no reliable response (data not shown). The morphology of type II sensillae also suggested that the adequate stimulus for them would be touching or direct bending by an external object. Such mechanoreceptive sensillae that are activated by direct bending are also well known in insects (Brown and Anderson, 1998; Gaffal and Theiß, 1978; Gnatzy and Hustert, 1989; Klein, 1981; Murphey, 1985).

Physiological characteristics of type II sensillae

Characteristic to the physiology of type II sensillae was that the sensory units associated with the sensilla did not respond reliably to mechanical stimulation: in the experiment shown in Fig. 7C, the maximal probability of response was approximately 0.7, indicating that the unit would fail to respond to the stimulus three times in every 10 cases. This unreliability might have been caused by inadequate stimulation in the present study: the twisted and bent structure of the type II sensilla (Fig. 4) made sure stimulation relatively difficult. The unreliability observed in the type II unit response might therefore reflect that of stimulation. The situation that the type II sensillae have a shape that is not suited for receiving point stimuli, however, holds true in the natural environment as well as in the laboratory. Thus, a sharp and pointed object in the natural surroundings of the cricket would not be able to effectively stimulate any single type II sensilla on the hindwing. Furthermore, also characteristic of the physiology of type II sensillae was that the timing of spike discharge in relation to sinusoidal stimulation of the sensilla was not precisely locked to the stimulus but considerably variable (Figs 7, 8). This variability might also be due to the difficulty in stimulation described above. But the difficulty would also be encountered by natural stimulation. The type II sensillae system would thus be unable to detect the precise direction of natural stimuli applied to the hindwing. These physiological characteristics of a single type II sensilla, not favourable for accurate detection of external stimuli, appeared to be compensated for by localized distribution of the sensillae on the hindwing tip. Even if several sensillae fail to respond, others could detect the presence of a specific object as far as it is not pointed but has some effective area for contact stimulation.

Although the response characteristics of the type II sensillae remain unknown at frequencies higher than 120 Hz (Fig. 7C), the present study suggests that the sensillae would not respond to slow or sustained stimuli (<1 Hz). Type II sensillae would thus operate as a low-cut filter but, apart from this function, they do not appear to be tuned for detection of any specific aspect of mechanosensory stimuli. The result that type II sensillae are generalists for detection of local mechanosensory stimuli rather than specialists for any particular stimulus parameters seems to be consistent with the result of behavioural analyses (Hiraguchi and Yamaguchi, 2000) that no specific stimulus profile was noticed in experimental elicitation of the hindwing-evoked escape jumping.

The finding that the type II unit showed a relatively slow conduction velocity (Fig. 6) appears to be inconsistent with the hypothesis that it is involved in escape jumping, which, in general, should be carried out as quickly as possible. It should be noted here, however, that the type II sensillae are activated by contact stimuli, i.e. pinching and touching (Hiraguchi and Yamaguchi, 2000). This is in contrast to the cercal sensillae, which are activated by distant stimuli, i.e. air current, to evoke escape running from the predator (Murphey, 1985). Hence, one possibility would be that the response time is not critical for escape jumping: it may be elicited in natural conditions by non-lethal stimuli such as biting by nearby conspecifics or hitting by soil lumps. The deflected shaft of type II sensillae (Fig. 4) would be advantageous to protect themselves from snapping against such mechanical stimulation. Further study is needed to test this possibility by careful observation of cricket behaviour in their natural habitat.

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