Varieties of filiform hairs: range fractionation by sensory afferents and cercal interneurons of a cricket

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Summary. 1. Wide variations in the size of the cercal filiform hairs in *Gryllus bimaculatus* are described (Figs. 1, 2). The length of the hairs varies from 30 to 1,500 μm, while the diameter varies from 1.5 to 9 μm (Fig. 2). The range of hair length overlaps well with the physical depth of air-motion on a substrate floor. The length dependency of sensory threshold to air-current stimulus is predictable.

2. The sensory threshold to the alternating air-current stimulus was measured. The sensory afferent was penetrated at the cercal nerve bundle. The length of the filiform hair of the recorded afferent was identified by needle probe. All sensory afferents showed phase locked responses to each cycle of bursts of sinusoidal air-current (Fig. 3).

3. The long filiform hairs are spontaneously active and sensitive to a low frequency stimulus (Figs. 3, 4). They are regarded as velocity sensitive hairs. The short hairs are spontaneously inactive and insensitive to low frequency stimulus. They are acceleration sensitive hairs.

4. The selective deprivation of the sensory hairs longer than 500 μm has little effect on the threshold of large interneurons 9-1 (LGI) and 8-1 (MGI) (Fig. 6). Under the same deprivation we were unable to record small-sized interneurons 10-2 and 10-3.

5. The threshold curves of the sensory hairs and those of the cercal interneurons are compared (Fig. 7). The conspicuously long cercal filiform hairs converge upon two small sized interneurons 10-2 and 10-3. Large cercal interneurons 9-1 (LGI) and 8-1 (MGI) receive the main excitatory sensory input from the short hairs around 200–300 μm.

Introduction

Orthopteran and blattellan insects bear a pair of cerci at the rear end of the body. The cerci have a large number of mechanoreceptive hairs which have been thought to be responsible for the evasion reflex against air disturbance (Camhi and Tom 1978; Camhi et al. 1978; Bentley 1975; Bentley and Hoy 1974). Seven cercal interneurons have been identified in the cricket, *Gryllus bimaculatus* (Kanou and Shimozawa 1984). In terms of the air-particle velocity threshold, the interneurons show a variety of frequency dependencies; interneurons 10-2 and 10-3 are velocity sensitive, 8-1 (MGI), 9-1 (LGI) and others are acceleration sensitive.

The question arises whether this variety of response properties of the interneurons results from neuronal information processing of input from a uniform sensory afferent, or from a physical range fractionation in the mechanoreceptors. Petrovskaya et al. (1970) and Counter (1976) have demonstrated that there are cercal mechanoreceptors with different tuning curves to tone pulse, therefore we expected the latter case.

Air behaves as a viscous fluid under the conditions to which the dimension and frequency of cercal hairs are subject. Therefore the cercal hairs receive principally a kind of drag-force from the air-disturbance (Tautz 1979; Shimozawa and Kanou 1984). The viscosity of air provides not only the drag-force to a hair but also a stagnating force to air-mass on the cuticular substrate. This stagnating air-mass is called the boundary layer. The thickness of the boundary layer varies with the frequency (Schlichting 1979). The length of sensory
hair must therefore affect its threshold and the frequency dependency. In this paper, we will show, (1) that the cricket has a variety of lengths of filiform hairs on the cerci; (2) that the cercal hairs of different lengths have different frequency-threshold curves; (3) that the removal of long cercal hairs has a minor effect on the thresholds of the acceleration sensitive interneurons.

The aerodynamics and other mechanical properties which affect the response properties of the sensory afferents are dealt with in the companion paper (Shimozawa and Kanou 1984).

Our results show that large cercal interneurons such as LGI (9-1) and MGI (8-1) do receive their main excitatory input from rather short cercal hairs but not from the conspicuously long filiform hairs. The long hairs connect to two small sized interneurons: 10-2 and 10-3.

Material and methods
We used both sexes of the field black cricket (Gryllus bimaculatus De Geer), 1–2 weeks after the imaginal ecdysis. Animal rearing and the physiological saline solution were the same as in the previous paper (Kanou and Shimozawa 1984).

Morphology. The size of the cercal filiform hairs and cuticular structures were examined on the CRT screen of a scanning electron microscope (SEM, JEOL T-200). Cerci were dissected, mounted on the specimen stage after an acetone wash and air drying, and sputter coated with gold. Some cerci were directly gold-sputtered without an acetone wash in order to see whether small structures are covered with biogenic wax.

Threshold measurement. The prothorax, wings, legs, and ovipositor were removed from the abdomen. The specimen was pinned on a cork board dorsal side up after sealing the cut ends with paraffin. The abdominal tergites and viscera were removed so as to expose the terminal ganglion and cercal nerve. The body cavity was filled with a saline solution. The cercal motor nerves were cut out in order to prevent a reflex movement to a probe needle (see below). The reflex movement of the cercus resulted in an electrode dislocation. In order to improve the electrode penetration into a sensory axon, the ganglion sheath was torn with fine forceps for certain lengths of cercal nerve; alternatively, 70–100 µl of 1% Trypsin (Sigma, Type III, dissolved in saline) was added to the body cavity. After 5–8 min, the same amount of Trypsin inhibitor (Sigma, Type II-S, 1% solution) was added.

Glass microelectrodes of 30–50 MΩhm, filled with 2 mol/l K-Acetate or 3 mol/l KCl, were used. The microelectrode was aimed at the cercal nerve at least one third of its length from the terminal ganglion. The intracellularly or quasi-intracellularly recorded signals were fed into a microelectrode amplifier and displayed on a CRT in the conventional way. The spike height of the recorded signal ranged from 4 to 80 mV.

The air-current stimuli were applied to the cercus and returning to the same phase after completion of a number of cycles (Fig. 3). This wave form was generated by a phase-controllable function generator in trigger mode. The alternating frequency of air-current was chosen in the function generator. The number of cycles comprising a burst varied with frequency. At least one complete cycle was given for the frequencies lower than 5 Hz. The amplitude of the electrical signal fed into the moving coils was controlled by a step dB attenuator. The stimulus intensity of the wind tunnel was calibrated in terms of peak velocity of the sinusoidal air-current. Details of calibration were described in the previous paper (Kanou and Shimozawa 1984).

Measurements of the minimum intensity of air-current for response at a variety of frequencies gave a frequency-threshold curve. Two stimulus parameters, peak velocity and alternating frequency, were controlled separately while the threshold was being searched for.

The difference in discharge of sensory afferent to ± 1 dB change of stimulus intensity at threshold was rather acute (see below about stimulus intensity). The threshold at a stimulus frequency was therefore determined within 2 dB accuracy (1/10 of the logarithmically scaled division in Figs. 4, 5, 6, 7). Even in the spontaneously active afferent, the difference to ± 1 dB change at threshold was still acute; the phase-locked response (Fig. 3) was clearly recognizable as a frequency-modulated sizzling sound when heard through an audio-monitor. In addition to the audio-monitor, if a difficulty was encountered, we superimposed 5–10 records of response on a screen of a storage oscilloscope (Tektronix 5115). The oscilloscope sweep was synchronized with the stimulus so as to deliver the stimulus at the middle of screen. The response, if any, was readily detected by a density-modulation of spikes at a certain phase of the stimulus.

After threshold measurements at different frequencies, the filiform hair responsible for the recorded afferent was searched for with a fine tungsten needle (probe) under a dissection microscope. Observing the responses to a suprathreshold air-current stimulus, filiform hairs were touched with the probe, one by one. The single filiform hair of the recorded afferent was identifiable because the response to the air-current stimulus ceased after a vigorous discharge to probe-touching. If filiform hairs make contact with other objects, an electrostatic or other kind of cohesive force prevents the hair from moving with the air-current. The length of the identified filiform hair was measured with a calibrated ocular micrometer, only when the hair stood perpendicularly to the microscope axis (vertical).

The directional sensitivity of the threshold was not measured because of the difficulty in preventing electrode dislocation during the tunnel movement. The air-current was parallel to the cricket's body axis. The angle of cerci was about 30° from the body axis.

Although the preparation was placed in the wind-tunnel, the sensory afferents responded to the audio-monitor sound and a bowling occurred. To prevent this, the experimenter had to wear an ear-phone for activity monitoring.

Threshold measurement of the cercal interneuron and morphological identification. The axon of an interneuron was penetrated at the connective just anterior to the terminal ganglion in a...