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The antennal feathered hairs in the crayfish: a non-innervated stimulus transmitting system

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Summary. Mechanical stimulation of feathered hairs on the crayfish antenna elicits spike activity in nerve bundles running in the flagellum. Electron microscopical studies showed, however, that these hairs are not innervated. Instead these hairs can be coupled mechanically with nearby innervated hairs of a different type, which perform the sensory transduction.

Introduction

In a previous paper Tautz et al. (1981) reported on morphological and physiological properties of two types of hairs found on the flagellum of the second antenna of the crayfish *Astacus leptodactylus*. They described smooth upright hairs responding to movement of the surrounding medium and to touch. A second feathered type of hair, unusual in that it lies pressed against the antenna bridging the articulation between adjacent segments, can elicit an electrophysiological response when touched directly or displaced by bending the flagellum (Tautz et al. 1981). Analysis of 1 µm sections suggested that each feathered hair was innervated by two sensory cells.

In the present paper we report on electron microscopical findings that require a new interpretation with respect to the role of the procumbent feathered hairs in stimulus transmission.

Material and methods

The investigations were carried out on adults of *Astacus leptodactylus*.

Electrophysiological experiments. The experiments were performed as described already in Tautz et al. (1981). The activity of the sensory cells was recorded extracellularly from nerve bundles exposed in the proximal flagellar segments. The hair attached to the sensory cell recorded from was identified by touching hairs individually and looking for the strongest response. In order to find out if this sensory cell could also be activated by other mechanical stimuli a variety of mechanical manipulations were carried out: (i) The whole flagellum was bent in different directions, (ii) hairs of both types standing next to the identified hair were displaced, (iii) the soft membrane ('joint') connecting the annulus bearing the hair with the next distal annulus was touched slightly. Quantified mechanical stimuli were applied using stiff mechanical coupling of the whole flagellum or a single hair to a loudspeaker.

Laser-Doppler-vibrometry (LDV). Details of the LDV and the data evaluation by FFT (fast Fourier transform) analysis can be taken from Tautz et al. (1981). The LDV was done on the following preparation: Short pieces of the antenna, consisting of a few segments, were separated from the mid-flagellum and glued with the proximal end into a fitting glass-tubelet (Fig. 2b). This preparation was submerged under water. The most distal feathered hairs, in the intact antenna lying attached to the next distal segment, extended in the preparation beyond the edge of the segment thus offering the possibility of a controlled forced movement of the hairs. The feathered hairs were moved by direct coupling onto an accelerator (Brüel & Kjaer 4810). The direction of the deflection of the hair was perpendicular to the long axis of the flagellum and thus in the same direction the hair would be moved if deflected by bending of the intact flagellum.

Electron microscopy. In processing for transmission electron microscopy antennae were removed from animals, cut into several small pieces and then prefixed for 4 h at 20 °C in a mixture of 2–3% glutaraldehyde buffered to pH 7.4 with 100 mM Sørensen or 80 mM cacodylate buffer. The final osmolarity approximately 500 mOsm (osmolarity of the hemolymph of *Astacus:* 450 mOsm) was reached by the addition of sucrose. The tissues were postfixed for 2 h in 2% osmium tetroxide buffered to pH 7.4 with Sørensen or cacodylate buffer. The fixed pieces were dehydrated and embedded in Araldite. Sections about 120 nm thick were stained with lead citrate.

Results and discussion

The cuticular shaft of the procumbent feathered hairs bridging the intersegmental 'joint' is on average 900 µm long, shows a distally decreasing diameter and is hollow. The lumen of a fully developed hair is occasionally in part filled with intermingling fibers and cell fragments. The base of the hair, which is closed by a plug of polymorphous materi-

Abbreviation: LDV laser-Doppler-vibrometry

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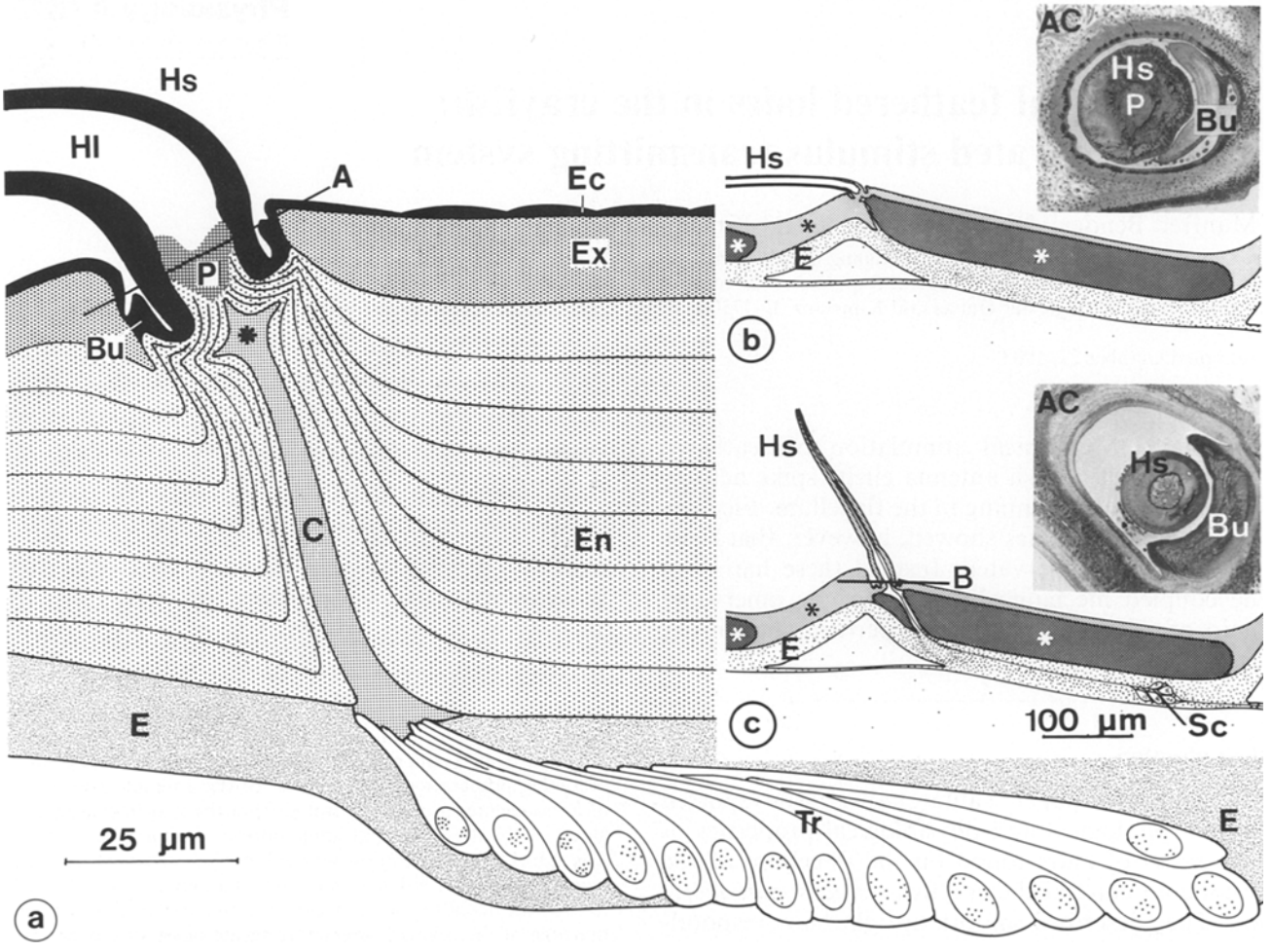

Fig. 1. a Schematic diagram of a longitudinal section through a fully developed procumbent feathered hair on the flagellum of the second antenna of *Astacus leptodactylus* (the hair shaft is not shown in its full length). Dilatation (asterisk) of the canal (*C*) within the annulus cuticle; epidermis (*E*), epicuticle (*Ec*), endocuticle (*En*), exocuticle (*Ex*), hair lumen (*Hl*), hair-shaft (*Hs*), plug (*P*), trichogen cells (*Tr*), bulge of the hair socket (*Bu*). **b** Highly schematic diagramm of a longitudinal section through a fully developed procumbent feathered hair. The canal leading to the base of the hair runs in a zone between uncalcified (black asterisk) and calcified annulus endocuticle (white asterisk). Epidermis (*E*). Inset: Cross section through the base of a procumbent feathered hair (the corresponding level A is indicated in **a**). The gap between hair-shaft (*Hs*) and surrounding annulus cuticle (*AC*) is less than 1 µm; plug (*P*), bulge of hair socket (*Bu*). 3000×. **c** Highly schematic diagram of a longitudinal section through a fully developed smooth hair on the flagellum of the second antenna of *Astacus*. It is noteworthy that in contrast to the procumbent feathered hair the canal (with the dendrites of the sensory cells) leading to the base of the hair runs completely through calcified annulus cuticle. Sensory cells (*Sc*). For other abbreviations see (**a**). Inset: Cross section through the hair base (the corresponding level *B* of the section is indicated in **c**); gap between hair-shaft (*Hs*) and surrounding annulus cuticle (*AC*) >3 µm. ×3000

al, is articulated into the cuticle of the flagellum annulus. The gap between hair-shaft and surrounding cuticle is less than 1 µm (Fig. 1b, Inset). A canal running between calcified and non-calcified annulus cuticle ends beneath the hair base (Fig. 1a, b). This canal is widened close to the hair base and ends near the plug (Fig. 1a). Within this canal no dendrites or a stimulus transmitting structure like a chorda (cf. Kouyama and Shimozawa 1982) were found. Up to 250–300 µm away from the hair base 11–14 cells associated with the hair could be identified (Fig. 1a). Sections of procumbent feathered hairs at varying intervals after ecdysis showed that up to one week after ecdysis small, 'dendrite-like' processes extend from these cells into the canal and hair lumen. About 4 weeks after ecdysis, however, these processes are no longer present (Fig. 1a). As the ultrastructural findings have shown these processes belong to trichogen cells, rather than sensory neurons as previously presumed (Tautz et al. 1981). The trichogen cells play an important role in secretion and shaping of the cuticular apparatus (e.g., the hair-shaft) of arthropod sensilla during the moulting processes (see Gnatzy 1978).

In contrary to the procumbent feathered hairs

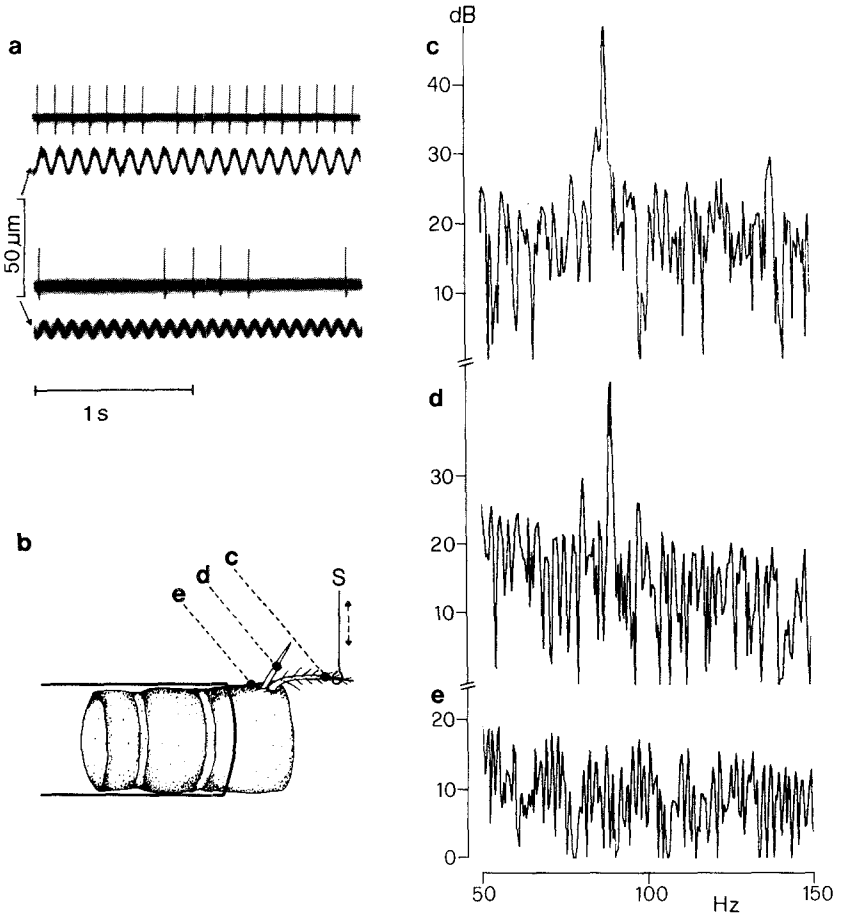

Fig. 2. a Extracellular recording from a non-spontaneous active mechanosensory cell attached to a smooth hair as (i) the smooth hair is moved directly (upper recording) or (ii) the smooth hair is cut off and a procumbent hair next to it is moved directly (lower recording). Lower traces: time course of stimulus. **b** Schematic arrangement for LDV. *S*, loop around hair connected to the vibrator. Arrow: direction of deflection (corresponding to Fig. 2c, d, e) points of measurements. **c, d, e** FFT-spectra measured on the procumbent hair (**c**), the smooth hair (**d**) and the annulus cuticle (**e** – see also **b**). In all 3 cases the procumbent hair was vibrated with 90 Hz. Velocity is plotted vs frequency; 0 dB = 1.5 µm/s

the smooth hairs on the flagellum are multiply innervated. Their 'scolopidial' organization (Bender 1983; see also Gnatzy and Schmidt 1982) is the morphological evidence that in fact at least one of the sensory cells is mechanosensitive.

Even though the procumbent feathered hairs are not innervated, sensory cell activity can be elicited by displacing them. This finding raises the question of which sensory structures may be stimulated by the procumbent-hair movement and how. It could be shown that the same unit responding to deflection of a smooth hair can also be stimulated by displacement of a neighboring procumbent hair even if the smooth hair is cut off at the base (Fig. 2a). Also bending the flagellum in different directions elicited this response. However, in some cases the mechanosensitive cell(s) belonging to a smooth hair could not be stimulated by the latter stimulus. In this context the distance between feathered and smooth hairs seems to be important. If the distance is less than about 5 µm movement of the procumbent hair leads to movement of the smooth hair as could be shown by LDV (Fig. 2c–e). However, the fact that the sensory cell could also be activated after cutting off the smooth hair makes it possible that the force is transmitted as stress and strain in the cuticle itself. The canal leading to the base of the procumbent hair runs in the zone between calcified and non-calcified annulus cuticle (see Fig. 1b). Here the mechanical elasticity of the cuticle can be expected to show the steepest gradient, and deformation might be transferred here most effectively.

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