Long, smooth hair sensilla on the spider leg coxa: Sensory physiology, central projection pattern, and proprioceptive function (Arachnida, Araneida)

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Summary. Rows of long, smooth hair sensilla situated on both sides of the leg coxae were examined in the spider *Cupiennius salei* (Ctenidae). The hair shafts point into the space between adjacent legs and are deflected when the hairs of one coxa touch the cuticle of the neighboring coxa. 1. Unlike the serrated hair shafts of the ubiquitous tactile and chemosensitive setae of spiders, these hairs are entirely smooth. At their base they are articulated in a socket with an asymmetrical groove that determines the direction of hair deflection. Hair shafts are up to 1000 μm long. The exact grouping of smooth hairs in rows is typical of the coxae for each pair of legs. 2. Unlike the other, multiply innervated cuticular sensilla of spiders, smooth hairs are supplied by only a single mechanosensitive neuron. This is confirmed by electrophysiological recordings from single hairs. Threshold deflection to elicit a spike response lies near 1°. The response to maintained, step-like stimuli declines rapidly. 3. All central endings of these hair receptors in the fused segmental ganglia are confined to dorsal neuropil of the ipsilateral neuromere. The specific arborization pattern resembles an elongated, three-pronged fork with a long central prong. Topography, natural stimulus situation, and the "phasic" response characteristic of smooth hairs suggest that spiders use these sensilla to monitor the relative distance between leg coxae during locomotion.

A. Introduction

Spiders have various cuticular mechanoreceptors such as the slit sensilla, the coxal hair plates, and long serrated setae bridging the leg joints which serve as proprioceptors; in addition each joint is supplied with internal receptors (for a review see Seyfarth 1985). We have now discovered rows of long, "smooth" hair sensilla on the coxae of spider legs that differ from the other, well-known tactile hairs. Their long, non-serrated hair shafts project into the space between adjacent legs; they are deflected and hence stimulated when two coxae come into contact with each other. Here we describe the external morphology and topography of the smooth hair sensilla in *Cupiennius salei* (Keyserling, 1877) and report on their sensory innervation, electrophysiological response, and specific afferent terminations in the fused subesophageal ganglia.

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B. Materials and methods

Animals. We used males and females of the large Central American spider *Cupiennius salei* (Keyserling, 1877) (Ctenidae). Adults (older than 9 months) or juveniles (5–6 months old) came from a colony bred in our laboratory.

Measurements. Observations on the topography of smooth hairs, on natural stimulation during leg movements, and measurements of hair lengths were made under a dissecting microscope. Scanning electron micrographs of air-dried specimens were taken with a Hitachi S-500 instrument after conventional coating with gold.

Hair stimulation, electrophysiological recordings. Sensory activity of individual hair sensilla was recorded extracellularly. Electrolytically sharpened tungsten needles were inserted into the leg cuticle ca. 20 μm proximal to the hair socket (similar to methods first described by Thurm 1962); the preparation was grounded with a reference electrode in the opisthosomal hemolymph space. Controlled deflections of single hairs were applied with a piezoelectrical device as described by Seyfarth et al. (1986, and in prep.).

Cobalt backfills. To fill the short axons, sensory cell somata, and dendrites of coxal hair sensilla, conventional methods for *retro*grade backfilling with cobalt (Altman and Tyrer 1980) were modified. Under deep CO_2-anesthesia, all leg nerves were cut close to the subesophageal ganglion complex and the central nervous system (CNS) removed through a sternal opening. The prosomal cavity was first filled with distilled water for 2 min, which was then replaced with a 5% solution of $CoCl_2$. The remaining coxal and prosomal musculature actually sealed off the main leg-nerve bundle against more distal leg parts, so that cobalt solution entered the sensory axons via the cut nerve ends.

To trace the central projections of single hair sensilla into the subesophageal ganglia, selective *antero*grade cobalt fillings were carried out in vivo as described by Tyrer et al. (1979) and Seyfarth et al. (1986, and in prep.). The preparations were silver-intensified according to conventional methods (Bacon and Altman 1977). Drawings of single, afferent projections were reconstructed from 30-μm serial sections. Well-known cobalt stainings of serrated tactile hairs (Eckweiler and Seyfarth 1988) and of coxal hair-plate sensilla (Seyfarth and Hammer 1988) located near long smooth hairs were used to confirm the specificity of the

new projection patterns described here. They also served as controls against possible misinterpretations due to accidental parallel fillings of other afferents.

C. Results

1. External morphology and topography of smooth hair sensilla on the coxa

The anterior and the posterior sides of the leg coxae possess 6 to 11 smooth hairs each, and each segmental leg pair has its typical pattern of hair topography. Direct observation under a stereomicroscope reveals that smooth hairs point into the space between adjacent legs and are deflected when the hairs of one coxa touch the cuticle of the neighboring coxa. Cuticle areas on the adjacent leg coxa, where contact takes place, are entirely hairless (asterisk symbols in Figs. 1; 2a, b).

A brief examination of some other spider species [*Nephila clavipes* (Linné, 1767), *Araneus* sp., *Dolomedes triton* (Walkenaer, 1837), *Isopoda immanis* (L. Koch, 1867), *Heteropoda jugulans* (L. Koch, 1876)] showed that they also possess rows of smooth coxal hairs very similar to those described here for *Cupiennius salei*.

Smooth hairs differ from the other types of hair sensilla in spiders in that their hair shaft appears entirely unstructured and smooth even at high magnification in the scanning electron microscope (Fig. 2e, f). On the anterio-ventral surface of the coxae smooth hairs are arranged in rows that run roughly parallel to the longitudinal ridge formed by a cuticular apodeme (Fig. 3a). Within each row, hair length declines from proximal (shaft length of ca. 1000 μm) to distal (shafts ca. 40 μm long). On the other, i.e., the *posterior* side of the coxae, smooth hairs are arranged in

rows that are oriented roughly perpendicular to the long leg axis; here the dorsalmost hair is the longest in each group. The posterior aspect of the hindleg coxae, i.e., the leg surface facing the opisthosoma, lacks smooth hair sensilla (Fig. 3b), nor are the pedipalpal coxae supplied with smooth hairs.

In their resting position hair shafts arise from the cuticle surface at an angle of 60°–70° (Fig. 2d). Hair shafts are articulated at their base in a cuticular socket. A groove on one side of the socket determines the direction of hair shaft deflection (Fig. 2e). The orientation of these socket grooves corresponds exactly to the direction in which the hairs are deflected upon leg contact (see Discussion). Fig. 3 (*right*) shows a schematized view of hair-shaft lengths and socket orientation.

2. Innervation

Our retrograde CoCl$_2$-impregnations reveal that the smooth hairs are innervated by a single bipolar sensory cell (25 sensilla were filled in three animals). The dendrite is ca. 100 μm long and inserts at the base of the hair shaft. The soma of the sensory cell is spindle-shaped; its long axis is up to 50 μm long.

Figure 3 (*left*) shows the arrangement of smooth hair sockets, sensory cell somata, and their axons in the coxa of leg R3. On the anterior side (Fig. 3a), axons from smooth hair sensilla merge into sensory nerve *aCo*. The nerve includes axons from other coxal sense organs and enters the subesophageal ganglion separately from the main leg-nerve bundle. Axons from smooth hair sensilla lying on the posterior coxa (Fig. 3b) run in sensory nerve *pCo* that merges into the main leg nerve near the sterno-coxal joint.

3. Electrophysiological response

The sensilla are not active in their resting position. The stimulus amplitude to elicit 1–2 spikes lies at deflection angles of 1°–2° (step stimuli, rise time of 10 ms). Only deflections in the direction morphologically determined by the socket groove excite. This was confirmed in several cases by first recording from a sensillum, noting its directional sensitivity, and then examining the same hair and the orientation of its socket groove under the scanning electron microscope. Only one sensory unit responds over the entire possible deflection range; there is no sensory spiking activity during the (passive) return of the hair shaft to its resting position (Fig. 4a, b, right).

When long, maintained deflections are applied to the hair shaft, the spike frequency declines rapidly and the sensory cell ceases firing. Figure 4b shows a typical example; at a step-like deflection of 17.5° all spike activity ceases within the first 4 s of the 10 s-long stimulus. This "phasic" response pattern is unlike the adaptation behavior of hair plate sensilla; these are located next to the long smooth hairs studied here and show sustained, "tonic" spiking activity at comparable stimulus intensities for longer than 10 s (Seyfarth et al. 1986, and in prep.).
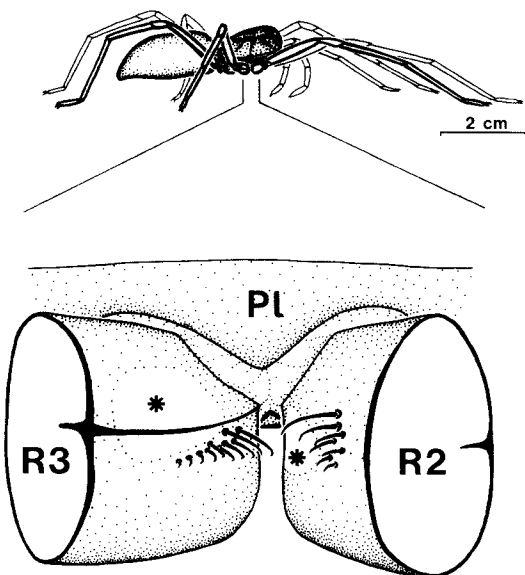

Fig. 1. Position of long, smooth hairs and of smooth cuticle surfaces on adjacent leg coxae. Top: Lateral view of *Cupiennius salei*. Bottom: Enlarged view into space between third (*R3*) and second (*R2*) leg coxae. Row of smooth hairs on anterior side of *R3* runs approximately parallel to longitudinal ridge of coxal apodeme while hairs on the posterior side are arranged in a row almost perpendicular to the long leg-axis. Both groups of hairs lie opposite a spot of smooth cuticle (∗) on the adjacent coxa. *Pl*, pleural sclerite of spider prosoma

4. Central projection pattern

Selective anterograde cobalt fillings from smooth hair sensilla into the subesophageal ganglion reveal a typical branching pattern that – in dorsal view – resembles an elon-
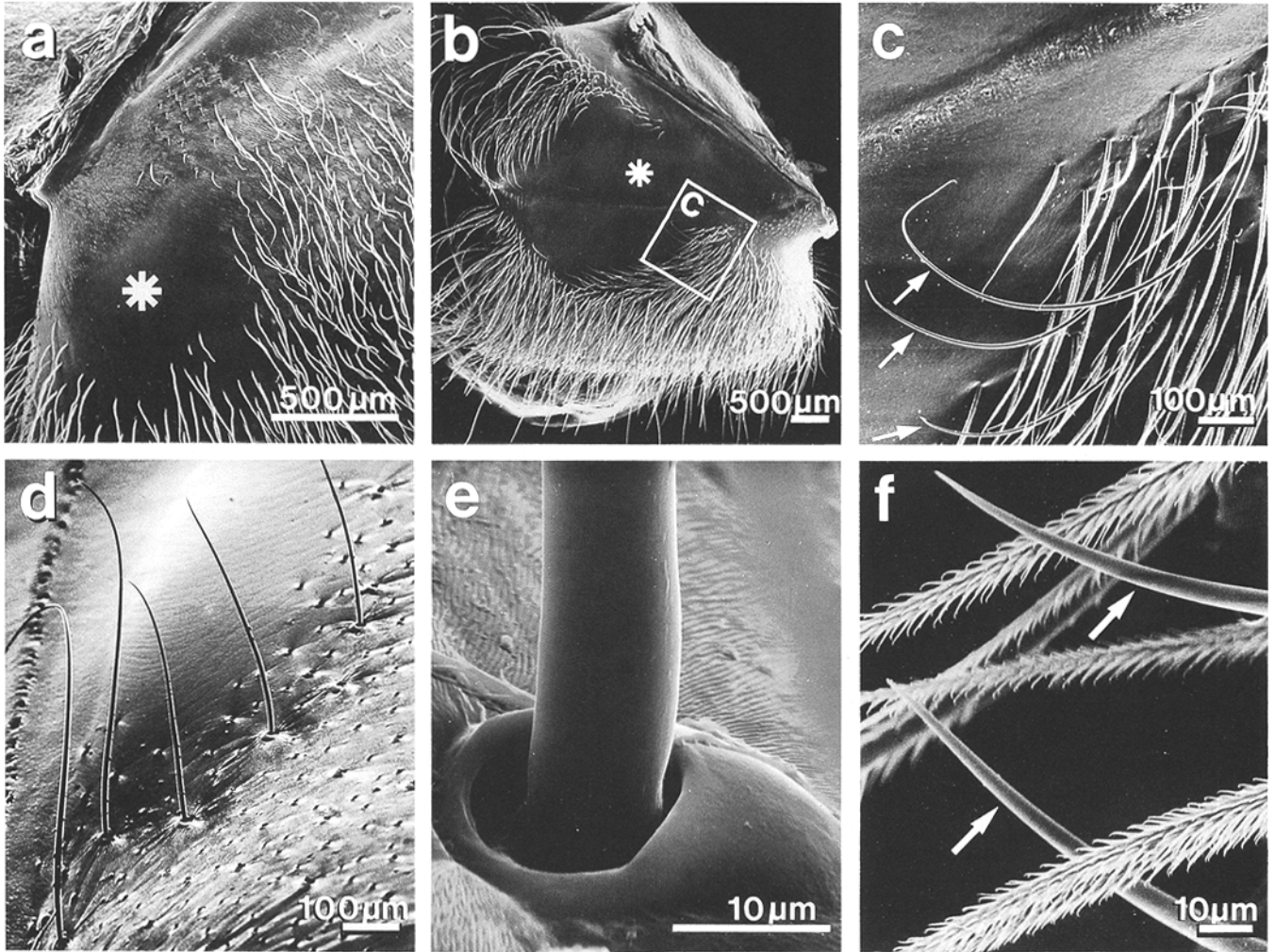
Fig. 2a–f. Scanning electronmicrographs of prosomal/coxal joint region and external hair structures. **a** Proximal part of posterior leg coxa (*leg R2*) with large spot of hairless, smooth cuticle (∗) that lies opposite long hairs of adjacent anterior leg coxa (i.e., of *R3*). **b** View of the other (anterior) aspect of the coxa with similar spot of hairless cuticle. White frame: area that is enlarged in **c**. **c** The three longest smooth hairs (*arrows*) surrounded by shorter serrated bristles on the anterior coxa. **d** Row of smooth hairs on anterior coxa after removal of serrated bristles. **e** Asymmetrical hair socket; smooth hair shaft rises from cuticle surface at an angle of ca. 70°; socket groove determines direction of hair deflection. **f** The tips of two long smooth hairs (*arrows*) next to serrated bristles

gated, three-pronged fork (Fig. 5). All axonal endings are confined to the ipsilateral neuromere. The center prong of the fork is always longer than the two lateral ones. Typically in the case of anterior sensilla, the anterior prong is oriented along the anterior segmental septum (Fig. 5a) while in posterior hairs the posterior prong approaches the posterior septum (Fig. 5b). Both anterior and posterior sensilla ascend to and terminate in mid-dorsal neuropil near areas that were termed "motor association region" by Babu and Barth (1984).

D. Discussion

### 1. Comparison with other hair sensilla on the spider leg

The long, smooth hair sensilla on the leg coxae described here show several interesting peculiarities when compared with other trichoid sensilla of spiders.

1. The smooth hair shaft arises from the cuticular socket at the relatively steep angle of 60°–70°. Other "tactile" sensilla, ubiquitous on the exoskeleton of spiders, have finely serrated hair shafts that rise from the cuticle at much flatter angles (15°–30°) and hence do not project into the space between adjacent legs.

2. While the other, well described cuticular sense organs of spiders – such as the serrated trichobothria, tactile and chemosensitive hairs, and slit sensilla – are innervated by several sensory neurons (Foelix 1985; Seyfarth 1985), smooth hairs are supplied by only one bipolar, mechanosensitive cell. The directional sensitivity of the sensillum, morphologically determined by the socket groove, is adapted to the mechanical stimulus situation that results when two adjacent coxae touch each other (see below).

3. The simple innervation pattern of smooth hairs made it feasible to trace the central projections of the sensory neurons unambiguously into the subesophageal leg ganglion; all central terminations are clearly confined to the ipsilateral neuromere. By contrast, the projections of serrated,
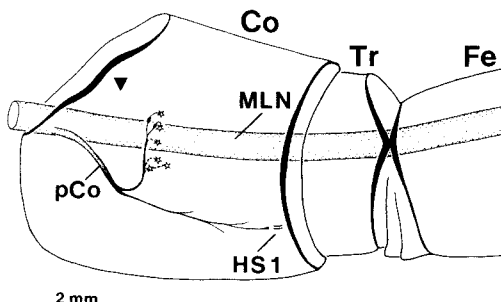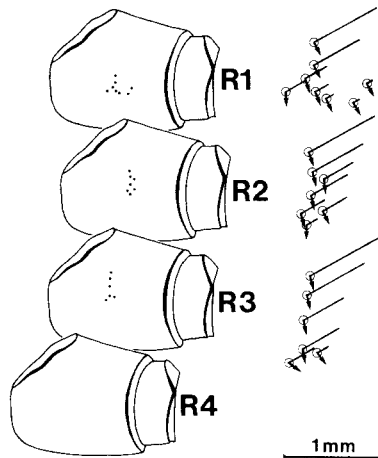
R3, anterior
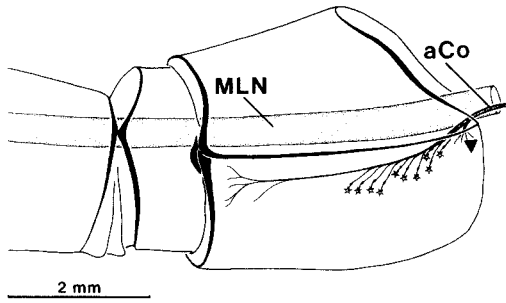
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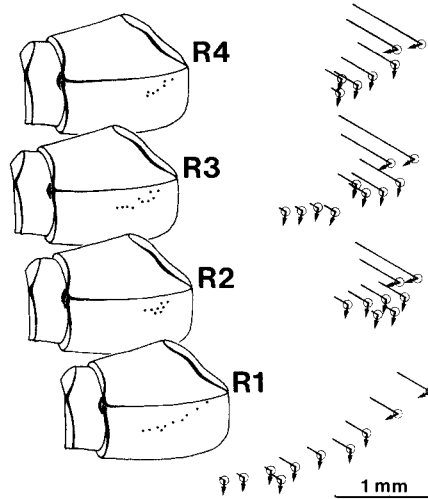
R3, posterior

b

Fig. 3a, b. Topography, shaft-lengths, and innervation of smooth coxal hairs. a anterior coxa b posterior aspect. Left: Innervation of hairs on coxa of *R3*; schematized drawing after retrograde cobalt-fillings; the hair sockets are indicated by *star symbols*. Center: Distribution of smooth hairs on legs *R1–R4*; hair sockets indicated by *black dots*. Note that there are no smooth hairs on posterior aspect of hindleg (*R4*). Right: Length distribution of hair shafts in each group (*solid lines*). For clarity all hair shafts are drawn schematically as if inserting at identical angles in their sockets. *Arrows* indicate the direction in which the hairs would actually bend when stimulated. ▼, position of coxal hair plates on both sides of the very proximal coxa; *Co*, coxa; *Tr*, trochanter; *Fe*, femur; *aCo, pCo,* anterior and posterior sensory nerve branch in coxa; *MLN*, main leg-nerve; *HS 1,* lyriform slit sense organ on posterior coxa
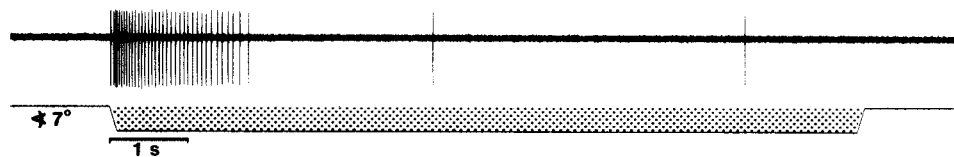
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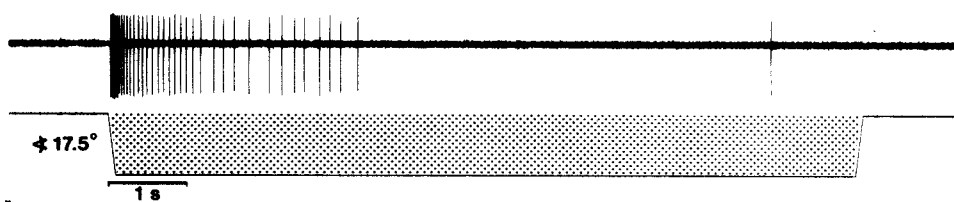
b

Fig. 4a, b. Electrophysiological response of smooth hair sensillum to step-like deflection from resting position. a, b Sensory discharge at two stimulus amplitudes (7° and 17.5°) maintained for 9.625 s (*stippled areas* above stimulus traces). Only distalward hair deflection (stimulus trace down) elicits a spike response that ceases within 4 s after stimulus onset
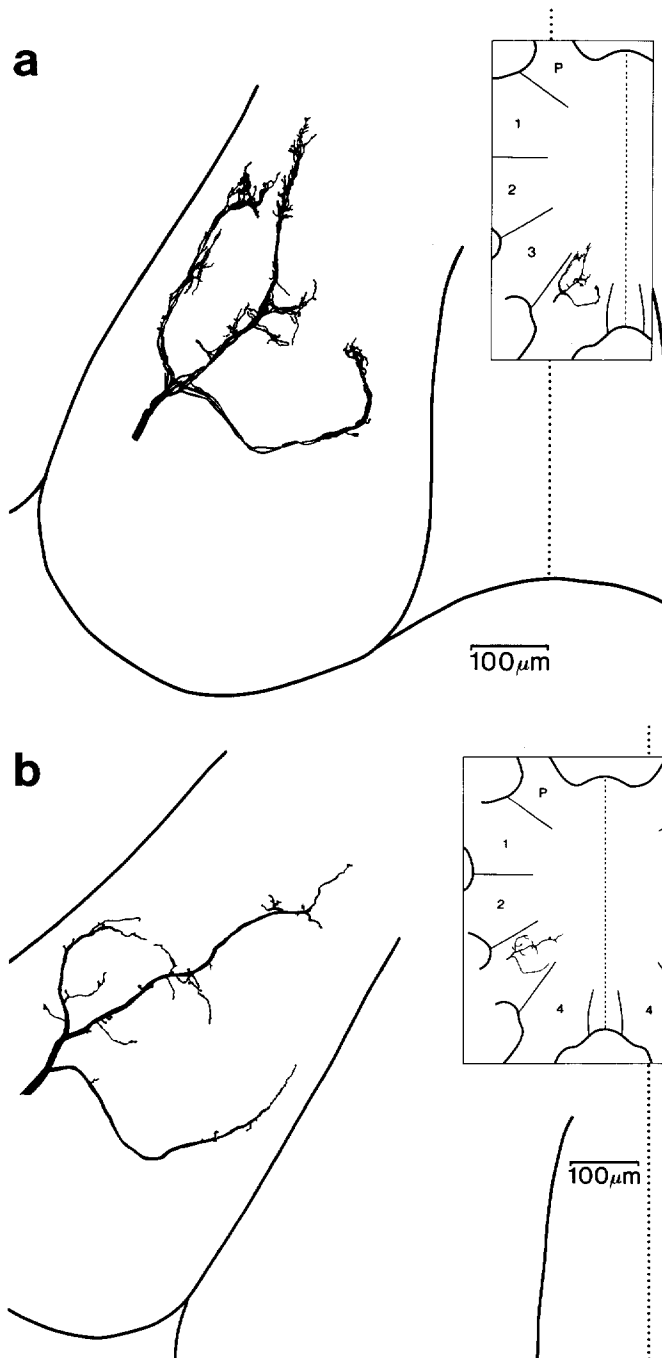
Fig. 5a, b. Central projection patterns of smooth hair sensilla in the fused subesophageal ganglion, dorsal view. Inset: schematized outline of left half of subesophageal ganglion complex; *dotted lines* symbolize longitudinal midline of CNS. **a** Projection pattern of three anterior sensilla in L4; **b** Axonal branching of two posterior sensilla in L3

tactile hairs (where unequivocal cobalt backfills are not possible because they are triply innervated) on the coxa extend into much more central regions of the fused ganglia; they reach into adjacent ipsilateral neuromeres and partially merge into plurisegmental, longitudinal tracts (Eckweiler and Seyfarth 1988; Seyfarth and Hammer 1988).

Smooth hair sensilla show certain similarities only to one other type of cuticular sense organ on the spider leg.

These are the specialized "hair plate sensilla" situated at the very proximal edge of the coxa, i.e., next to the articulation with the prosoma (see also Fig. 3, left). In *Cupiennius salei*, hair plate sensilla occur in clusters of 27–70 short hairs; their curved, smooth hair shafts are depressed when the pleural membrane rolls over them during coxal movements (Seyfarth et al. 1985). Just like the smooth hairs described in the present paper, hair plate sensilla are supplied by only one bipolar sensory neuron whose central projection pattern is also limited to the ipsilateral neuromere (Seyfarth et al. 1986, and in prep.).

2. Proprioceptive function

The topography, directional orientation, and electrophysiological response of the smooth hair sensilla appear to be perfectly adapted for signalling coxal contact of two adjacent legs. Direct observation under a stereomicroscope reveals that these hairs project into the narrow space between two coxae and are bent as soon as they touch the hairless spot on the adjacent, opposite coxa. Hair shaft deflection then follows the relative movement of the two coxae. Consider, for example, the smooth hairs on the *anterior* side of a middle leg such as R3 (see also Figs. 1, 2a). If R3 is moved forward against R2, the two long, proximal hairs are touched first and then bent distally. As R3 moves closer, its anterior surface rotates slightly upwards (due to the peculiar prosomal/coxal articulation), so that the row of short hairs is then deflected downwards. Conversely, if R2 is retracted against the adjacent third leg, initially the long posterior hairs touch the bald spot on coxa R3 and are then successively deflected downwards as the posterior surface of the R2-coxa rotates upwards (see Figs. 1, 2b).

Spiders walk with an alternating tetrapod gait in which adjacent legs are moved approximately in anti-phase (see the review by Seyfarth 1985). Leg protraction and retraction during stepping is accompanied by considerable rotation about the long leg-axis at the proximal joints (Kaestner 1924). The leg coxae are spaced so closely together at their articulation on the prosoma that they are likely to touch at each alternating stepping cycle. Perceiving coxal contact via smooth hair sensilla could thus play a role during locomotion, for instance in relative limb coordination.

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