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# Morphological Correlates of Differences in Pheromone Sensitivity in Insect Sensilla

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Abstract. *Scanning electron microscopy and single unit recordings of male* Trichoplusia ni *antennae reveal at least two classes of pheromone-sensitive sensilla trichodea. The longer sensillum contains two receptor neurons each with small amounts of spontaneous activity. One neuron responds to large (10-microgram) doses of (Z)-7-dodecenyl acetate, a component of the female sex pheromone. The shorter sensillum contains two receptor neurons both with larger amounts of spontaneous activity and increased sensitivity to low (0.01-microgram) doses of pheromone.*

The functional capabilities of sensory receptor neurons are ultimately determined by intrinsic surface membrane properties that account for the neurons' ability to detect particular environmental energies and extrinsic properties that serve to couple the sensory neuron to appropriate external stimuli. All of the intrinsic and extrinsic components of an adult olfactory sensillum arise from a single mother cell in the larval imaginal disk (*1*). We sought to determine in a single defined class of sensilla if the precise cell lineage patterns characteristic of development in olfactory sensilla might lead to distinctive morphological markers for differences in physiological properties.

Separate morphological and electrophysiological studies in a range of different insect species led us to suspect that, even in a restricted subset of the available olfactory sensilla, we would encounter a variety of differences among individual sensilla (*2*). Therefore, to relate a sensillum's particular morphology with the physiological properties of its receptor neurons, we examined individual sensilla electrophysiologically and labeled them unambiguously for subsequent morphological examination. We restricted our investigations to the sensilla trichodea on the antennae of the male cabbage looper (*Trichoplusia ni*, Hübner). These olfactory sensilla have long (15 to 60 μm) cylindrical cuticular shafts that taper apically from a socket diameter of approximately 2 μm. Each is innervated by two primary olfactory receptor neurons whose dendrites fill the lumen of the shaft and whose axons project, without synapse, to the deutocerebrum (*3*). These receptor neurons are specialized to respond to the pheromones produced by the female moth and are thought to provide the sensory input that allows the male to detect and locate the female. Usually the two olfactory receptor neurons within the sensilla trichodea can be differentiated from each other by the amplitudes of their action potentials, with the cell producing the larger spike designated A and the cell producing the small spike designated B.

Standard techniques were used to record extracellular action potentials from individual neurons (*4*). Stimulus application, action potential discrimination, and data analysis were accomplished with a minicomputer (*5*). Light microscopy (~ ×600) was used to determine both the sensillum from which recordings were to be obtained and the exact placement of the electrodes. After electrophysiological responses to a range of stimuli were acquired, a map of the surface of the antenna was sketched, and adjacent sensilla and scales were removed from the segment with the microelectrode. This produced a distinctive surface pattern that allowed subsequent identification of the recorded sensillum with scanning electron microscopy (SEM). Each sensillum characterized physiologically was easily identified by SEM with the landmarks created by sensilla removal (*6*).

In *T. ni*, pheromone-sensitive sensilla include at least two classes of sensilla trichodea that can be differentiated from each other by the spontaneous activity of their receptor neurons and the relative sensitivity of the neurons to pheromone (Fig. 1a). The first class has relatively high spontaneous activity (HS) averaging 1.39 impulses per second for the A neuron and 1.20 for the B neuron. The A neuron in HS sensilla is reliably excited by low doses (0.01 μg) of (*Z*)-7-dodecenyl acetate, a behaviorally active pheromone component produced by female *T. ni*. In contrast, the B neuron is reliably excited by low doses (0.01 μg) of

(*Z*)-7-dodecenyl alcohol. The second class of sensilla has relatively low spontaneous activity (LS), averaging 0.12 impulse per second for the A and 0.17 for the B neuron. The A cell is unresponsive to (*Z*)-7-dodecenyl acetate and (*Z*)-7-dodecenyl alcohol even when doses are increased 10,000-fold. The B cell, on the contrary, is reliably excited by doses of (*Z*)-7-dodecenyl acetate about 1000 times larger than those eliciting comparable responses in the A cell of the HS class. In every sensillum so far examined ($N > 120$), the correlation between the relative spike amplitudes of the two neurons, their relative levels of spontaneous electrical activity, and the dose dependence of their responsiveness to pheromones has been invariant.

When each electrophysiologically classified sensillum (HS and LS, $N = 47$) was examined with high-resolution SEM (×20,000), a number of morphological differences among them was observed. These differences included pore size, shape, and density; surface annulations; degree of taper and curvature; total length; and the relative distribution of the two classes over the antennal segment. For purposes of exposition, the difference in total length is most readily apparent. Typical differences in length between HS and LS are shown in Fig. 1b, which details the external morphology of the two sensilla from which the recordings in Fig. 1a were obtained. The average HS sensillum is significantly shorter (28.7 ± 2.6 μm, mean ± standard deviation) than the average LS sensillum (35.3 ± 4.2 μm), as can be seen in the sample distribution of Fig. 2. The two classes can be independently evaluated because most sensilla are easily differentiated according to length by standard light microscopy (*7*).

The identification of physiologically distinct groups of receptor neurons in sensilla trichodea of *T. ni* does not represent an isolated occurrence because similar differences have been noted in other species (*3, 8*). However, our observations show that even when only pheromone-sensitive olfactory sensilla are considered, they too may be subdivided into several distinct classes, each of which is distinguished by a particular combination of morphological and physiological properties. Receptor neurons in *T. ni* characterized by HS activity are more sensitive to pheromone components and are activated by relatively few of the compounds examined (*9*). Their sensilla are shorter, straighter, have more cuticular pores, and are preferentially obtained on the distal margins of each antennal segment. Receptor neurons characterized by LS activity are relatively insensitive to *T. ni* pheromone components and are activated by a larger number of related compounds. Their sensilla are longer, S-shaped, have fewer pores, and are more uniformly distributed across the antennal segment.

These correlations between structure and function may be related to the clonal development of the adult sensillum, which results in a high degree of relatedness between its various cellular components. Certain morphological features such as length or the number, size, and distribution of cuticular pores are clearly involved with the access of stimuli to the receptor neurons. Others, such as the distribution of the two classes of sensilla on the antennal segment, are likely related to pattern formation in the imaginal disk, where the fate and location of adult structures is fixed. The other structural variants that we observed may be important in the ultimate physiological properties of a receptor neuron, but their exposition appears to require more detailed knowledge of the relationship between the internal and external structure of the sensillum and its molecular topography.

The existence of different pheromone-sensitive sensilla, each with its own response properties, spectrum of sensitivity to pheromones, and morphological specializations may complicate interpretations of receptor cell activity and neural coding inferred from electroantennograms or behavioral responses (*10, 11*). However, we have shown that the physiological classes can be easily distinguished from each other by differences in

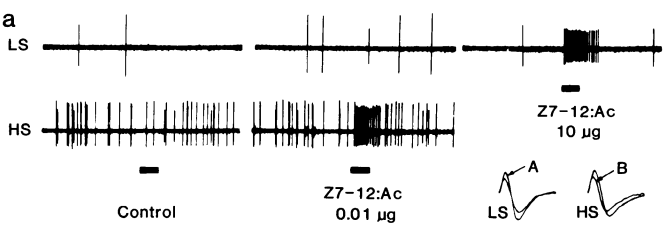



Fig. 1. Electrophysiological (a) and morphological (b) characteristics of pheromone-sensitive sensilla trichodea on male *T. ni* antennae. (a) The left section depicts typical spontaneous activities of the receptor neurons in a low spontaneous (LS) and a high spontaneous (HS) sensillum. The control was a 2-second puff of $O_2$-free $N_2$ through a cartridge loaded with 1 μl of light mineral oil, which was also used to dilute individual stimuli. The timing of stimulation is given by the bar in each section. The center section illustrates the responses to a cartridge loaded with 0.01 μg of (*Z*)-7-dodecenyl acetate (Z7-12:Ac), a component of the female sex pheromone. The upper portion of the right panel depicts the response obtained from the LS sensillum after stimulation with 10 μg of Z7-12:Ac. HS sensilla were not exposed to larger doses of pheromone because they change the sensillum's spontaneous activity and relative sensitivity. Insert at lower right illustrates typical differences in amplitude and waveform for the A and B spikes obtained from the LS and HS sensilla (each trace, 3.0 msec). (b) Low magnification SEM of the distal margin of segment 58. The labeled sensilla contained the receptor neurons from which the records in (a) were obtained.

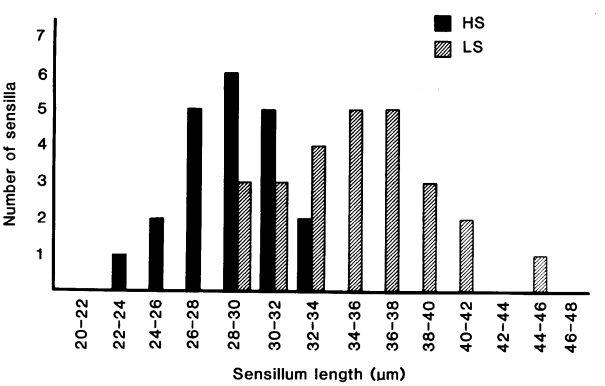


Fig. 2. A graph of the bimodal distribution of length for a sample of 47 physiologically defined (HS and LS) pheromone-sensitive sensilla trichodea. They were obtained on the distal segments (35 to 65) of male *T. ni* antennae. The lengths plotted are underestimates because it was rarely possible to position a stub so as to obtain a plane side view of the curved sensilla. Therefore, the distal one-third of a sensillum was usually out of the focal plane of the SEM and appeared foreshortened. This problem is more severe in the LS sensilla whose tips are normally recurved. These effects tend to increase the amount of apparent overlap between the HS and LS distribution. However, a *t*-test for groups with unequal standard deviations was significant ($t = 6.586$, d.f. = 20, $P < .001$, two-tailed).