A new *Trichoplusia ni* antennal receptor neuron that responds to attomolar concentrations of a minor pheromone component

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**Summary.** A newly discovered third class of sensillum trichodea on the antenna of *Trichoplusia ni* contains an olfactory receptor neuron that responds to (Z)-9-tetradecen-1-ol acetate at $1 \times 10^{-18}$ M. The threshold of this neuron is 100–1000 times lower than the thresholds of two previously described pheromone-sensitive neurons that detect (Z)-7-dodecen-1-ol acetate and (Z)-7-tetradecen-1-ol acetate. The sensitivity and selectivity of this receptor neuron is evidence that (Z)-9-tetradecen-1-ol acetate may be important for sexual behavior.

**Key words.** Sensillum; olfactory; (Z)-tetradecenyl acetate; insect.

An insect’s perception of a pheromone begins with detection of the pheromone by olfactory receptor neurons within different types of antennal sensilla. These receptor neurons encode the quality and quantity of the individual components in the pheromone blend, and project unsynapsed to a specialized neuropil, the macrogglomerulus. Integration of the information probably occurs in higher centers of the central nervous system. In *Trichoplusia ni* (Hübner), for example, a blend of six pheromone components is effective in eliciting sexual behaviors. Receptor neurons for two of these six *T. ni* female emission components have previously been reported, and here we describe a third class of receptor neuron. We describe its sensitivity and selectivity and briefly address pertinent questions about pheromone blend coding and detection. Two morphologically distinct *T. ni* antennal sensilla housing olfactory receptor neurons that respond to two of the components have been described previously. The first, designated the HS(a) neuron, responds to the most abundant pheromone component, (Z)-7-dodecen-1-ol acetate, which represents about 83% of the total pheromone emission. The second, designated the LS(b) neuron, responds to a less abundant component, (Z)-7-tetradecen-1-ol acetate, that comprises about 0.7% of the total. The neuron described in this report responds to (Z)-9-tetradecen-1-ol acetate, representing about 0.3% of the total pheromone emission. This neuron, originally found by Dr A. J. Grant (pers. comm.), is contained in a sensillum trichodeum that is usually located on the margin of the sensillar field at the proximal end of each flagellomere.

The insects were laboratory-reared, 1–5-day-old males held in a light cycle of 14 h daylight with lights off at 09.00 h and a relative humidity of about 80%. Details of the stimulus delivery device and recording procedures have been described in detail elsewhere. The insects were immobilized intact on a hollowed-out Plexiglas plate by securing their wings and legs with molten wax. The antenna was immobilized by strips of double-stick Scotch tape so that several flagellomeres were suspended on a strip of tape above a 3-mm diameter hole in the plate. The plate was secured on a support platform constructed so that the hole in the plate above which the antenna was suspended was connected to an exhaust. A glass stimulus delivery device was positioned over the antenna to provide an air stream that isolates the preparation from room air and delivers the stimulus. The small negative pressure from the exhaust entrained the isolation and stimulus air streams over the antenna. The air was commercially available bottled air (Linde, Inc.) filtered through charcoal, cotton and silica gel. Uninsulated sharpened tungsten electrodes inserted at the base of the sensillum coupled the electrical responses of the neuron to a Grass P 15D preamplifier. This signal was further amplified and led to a Digital® PDP 11/23 computer, where it was digitized, stored, and analyzed statistically.

The concentration of (Z)-9-tetradecen-1-ol acetate emitted from the dispenser was determined by capturing the volatile emissions from the dispenser in a capillary fitted with a post-trap tube that contained about 3 ml of hexane. At the end of a 3-min collection period the glass capillaries were rinsed with 500 μl of hexane. The quantity collected was measured by gas-liquid chromatographic (GLC) procedures that employed internal and external standards where appropriate. Emission levels below GLC-detectable levels were estimated by extrapolating a line parallel to that previously determined for (Z)-7-dodecen-1-ol acetate. The final concentration delivered to the antenna was obtained by dividing the emission rate by the 1200 ml/min room-air isolation air flow delivered through the stimulus delivery device. The (Z)-9-tetradecen-1-ol acetate was greater than 94% pure by GLC analysis and contained no high molecular weight impurities.

The antenna bears only a few sensilla trichodea with neurons sensitive to (Z)-9-tetradecen-1-ol acetate. Surveying different parts of the antenna, we found this type of sensillum most frequently on the lateral- and medial margins of the sensillar field at the proximal end of a flagellomere. More than two of these sensilla were rarely observed on any flagellomere and we seldom found it in
other locations. Because two action potentials (spikes) of non-overlapping amplitude were observed within the sensillum, we postulated that it contained two neurons. We measured the responses from neurons in 15 sensilla to the six most abundant pheromone components, (Z)-7-dodecen-1-ol, (Z)-7-tetradecen-1-ol acetate, (Z)-9-tetradecen-1-ol acetate, (Z)-5-dodecen-1-ol acetate, 11-dodecen-1-ol acetate and dodecan-1-ol acetate. The spontaneous activities also were measured to determine the neural threshold. All stimuli were presented in order of increasing stimulus intensity to minimize receptor fatigue and/or adaptation. The data, where appropriate, were analyzed by regression analysis, analysis of variance and/or Student’s t-test.

The neuron with the largest spike, designated ‘a’, exhibited a spontaneous activity averaging 0.64 ± 0.18 spikes/s while exposed to room air. The second neuron, exhibiting the smaller action potential, designated the ‘b’ neuron, did not respond reliably to any of the stimuli tested. Its spontaneous frequency is less than 1 per 1000 s. Both spontaneous activities were statistically greater than zero (p < 0.01, t-test). We originally assigned the mnemonic NS (‘new sensillum’) to this neuron for computer data file classification. Because its spontaneous frequency overlaps the spontaneous activity of the other two receptor cell groups on the antenna, the designation HS and LS for high and low spontaneous activities should also be considered mnemonics and we consequently retain NS as the designated mnemonic for this class of sensillum. The threshold of the ‘a’ neuron to (Z)-9-tetradecen-1-ol acetate nears 1 × 10⁻¹⁸ M (fig.). An average of 3.3 ± 0.8 spikes/s was elicited from 9 of 15 neurons at a concentration of 1.86 × 10⁻¹⁸ M (log₁₀ -17.73 M) and an average of 5.3 ± 2.1 spikes/s was elicited from 13 of 15 neurons at 1.69 × 10⁻¹⁷ M (fig., b). Both averages were statistically greater than zero (p < 0.01, t-test and were statistically greater than the spontaneous activity (p < 0.01, t-test). The relationship is presented in log-log coordinates because neural stimulus-response power functions are linear in such a coordinate system, but it is also instructive to compare the response curve in log-linear coordinates. In the inset to figure (a) the response becomes asymptotic at around 1 × 10⁻¹⁴ M when graphed in log-linear coordinates. At and above this concentration the maximum individual neuronal response never exceeded 76 spikes/s. The sensitivity appears to be approximately equal to the receptor neurons of Antheraea polyphemus (Cramer) for (E, Z)-6,11-hexadecadienyl acetate. It is about 100 times more sensitive than the HS(a) and LS(b) receptor neurons for (Z)-7-dodecen-1-ol acetate and (Z)-7-tetradecen-1-ol acetate, respectively.

To consider this sensitivity in relation to the stimuli that the moth encounters in the natural environment, we estimated the concentration of (Z)-9-tetradecen-1-ol acetate emitted from a pheromone gland into a 1-cm³ volume of an air stream at 45.7 cm/s. The estimated concentration of 6.8 × 10⁻¹⁴ M lies well above the neuron’s response curve asymptote. Stimulation with excised pheromone glands evoked a reliable response by the NS(a) neuron. Based on the threshold sensitivity of this sensillum to (Z)-9-tetradecen-1-ol acetate and the response to the gland, we suggest that the male can detect this compound about as far downwind as it can the major pheromone component, (Z)-7-dodecen-1-ol acetate.

The qualitative selectivity of the NS(a) neuron was investigated by exposing the above 15 neurons to the five other components identified in the female pheromone emission. These neurons were up to a million times less responsive to the other components than to (Z)-9-

\[ a \] Concentration- and dose-response curves of the NS(a) receptor neuron response to six concentrations of (Z)-9-tetradecen-1-ol acetate (note that the logarithm of a set of means is not equal to the mean of a set of logarithms). b Typical spike trains elicited by four representative concentrations; stimulus onset at 3.0 s, off at 6.0 (ordinate ticks 0.50 mV, abscissa, s). c A series of overlapping waveforms of several action potentials illustrate the temporal and amplitude characteristics of the spike (ordinate ticks 0.25 mV, abscissa ticks 0.1 ms).
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tradecen-1-ol acetate, although each elicited a response above \(1 \times 10^{-12}\) M. Because the emission concentration of the other components is lower than \(1 \times 10^{-12}\), it is not likely that the NS(a) neuron can detect naturally-emitted concentrations of (Z)-7-dodecen-1-ol acetate or any of the other pheromone components. Consideration of the sensitivity and the qualitative selectivity of the three known pheromone-sensitive neurons on the \(T. ni\) antenna leads to the question of what compounds comprise the effective pheromone blend perceived by a male \(T. ni\). From one perspective, the ‘pheromone’ may comprise any compound identifiable in the effluent of a virgin calling female which enhances the sexual response. From another, however, the pheromone may comprise only those components detectable by receptor neurons at physiological concentrations. Ideally, these two perspectives should predict the same pheromone blend, but at this time, they yield incomplete answers for \(T. ni\). Fourteen compounds have been identified from virgin female cabbage looper sex pheromone gland extracts and seven from their volatile emissions. Wind tunnel bioassays suggest that the seven emitted components affect behavior. Thus, by behavioral criteria, the \(T. ni\) pheromone is comprised of several components. Electrophysiological methods have revealed the presence of three, highly selective neurons that have the requisite sensitivity to detect naturally-emitted concentrations of three of the pheromone components. No sensilla have been found with the requisite sensitivity for (Z)-5-dodecen-1-ol acetate, dodecan-1-ol acetate and 11-dodecen-1-ol acetate. Thus, the electrophysiological evidence implicates three components of the volatile emission in intraspecific chemical communication. The eventual resolution of the differences between the neurobiological record and the behavioral record is an intriguing problem.

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7 Van Der Pers, J. N. C., Ent. exp. appl. 31 (1982) 255.
16 Mayer, M. S., and Mankin, R. W., unpublished.