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Differential Adaptation Rates in a Male Moth's Sex Pheromone Receptor Neurons

B. S. Hansson and T. C. Baker*
Department of Ecology, Lund University, S-223 62 Lund

The behavior of a male moth approaching a source emitting the sex pheromone blend of the conspecific female has been extensively studied in many species of moths from different families (e.g., [1, 2]). A common feature in males of several of the species is that they will arrest their upwind progress to a pheromone source when an excessive amount of the pheromone blend is emitted [1, 3]. One species exhibiting this arrestment behavior is the turnip moth, *Agrotis segetum*. Female turnip moths produce three main pheromone components, (*Z*)-5-decenyl acetate (Z5-10:OAc), (*Z*)-7-dodecenyl acetate (Z7-12:OAc), and (*Z*)-9-tetradecenyl acetate (Z9-14:OAc). They also produce a con-

* Present address: Department of Entomology, University of California, Riverside, California 92521, USA

siderable amount of decyl acetate (10:OAc) [4, 5]. Male moths detect the female sex pheromone by olfactory receptor neurons located in hair-like structures, sensilla trichodea, on their antennae [6]. Male turnip moths possess three physiologically different sensillum types, each one containing a cell specialized for reception of one of the three major pheromone components.

In the Swedish population of this species, 66% of the cells are specialized for Z5-10:OAc, 33% for Z7-12:OAc, and only 1% for Z9-14:OAc [5, 7]. When presented with a pheromone plume in a wind tunnel, the males of this species would readily fly to a 1:5:2.5:0.6 mixture of the four components in the order as mentioned before [3]. Maximum upwind flight and source contact were obtained with 3 and 30 μg of Z5-10:OAc, and the other components in their above-listed proportions loaded on rubber dispensers. A 0.3-μg loading evoked somewhat diminished levels of upwind flight, but a high percentage of source contact occurred by those males that did fly upwind. However, the 300-μg loading caused source contact to drop to virtually zero [3], while evoking high levels of upwind flight initiation. Several hypotheses have been proposed to explain such arrestment, but an underlying cause has only recently been suggested. In an earlier paper we demonstrated that in *A. segetum* there is a close correlation between the virtually total adaptation of the male olfactory receptor neuron for Z5-10:OAc, and the arrestment of upwind flight [8]. We also demonstrated that in this species, the concentration causing such adaptation is the same as the one causing arrestment. In contrast, in *Heliothis virescens,* a species in which we were unable to evoke concentration-dependent arrestment, it was not possible to adapt the male pheromone receptor neurons [9].

The present paper presents a finer-grained analysis of differential adaptation in *A. segetum,* including an examination of the adaptation rates of all three different physiological receptor neuron types when placed in a pheromone plume 70 cm downwind of the source. All three receptor neuron types showed a typical spontaneous activity of $\leqslant 1$ impulse/s (Figs. 1, 2).

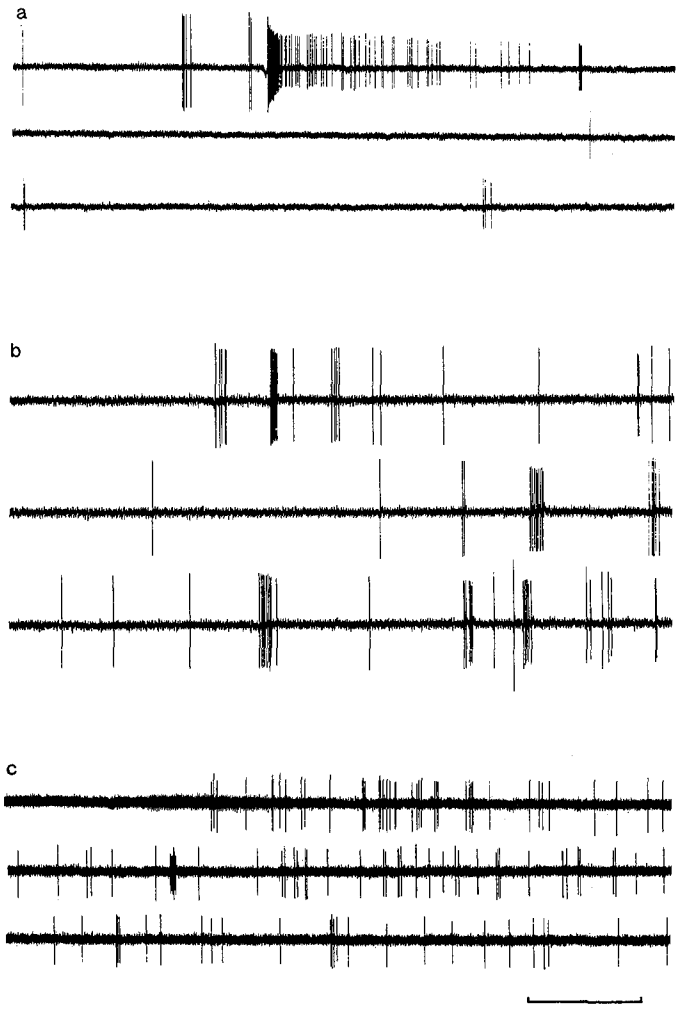

Fig. 1. Responses of the three physiologically different pheromone-sensitive cell types on a male *A. segetum* antenna, when challenged with a plume from a source loaded with the highest, 300 μg, blend of components. The first stimulus molecules hit the antenna after approximately 3s. a) Z5-10:OAc-sensitive cell, b) Z7-12:OAc-sensitive, c) Z9-14:OAc-sensitive, *scale bar* 1 s. The three traces for each cell respresent a continuous recording. Male *A. segetum* were investigated at an age of 2 – 3 days. Responses from single olfactory sensilla were recorded by means of the tip recording method [6, 18]. The antenna was excised from the animal and placed in a grounded pipette electrode. The tip of a sensillum trichodeum was cut by means of two glass knives, and a recording electrode was placed in contact with the cut surface, making contact with the neurons situated inside the sensillum. The recording electrode was a pipette electrode filled with saline. The stimulus was a mixture of pheromone components known to be behaviorally active in *A. segetum,* 1:5:2.5:0.6 ratio of Z5-10:OAc, Z7-12:OAC, Z9-14:OAc, and 10:OAC [4, 5]. The mixture was diluted in four decadic steps, such that rubber septa dispensers were loaded with 0.3, 3, 30 and 300 μg of Z5-10:OAc, respectively, and the other compounds in relation to this, exactly the same concentrations used in earlier behavior wind tunnel studies. A miniature wind tunnel, 80 cm long, 20 cm i.d. was used to form the plume structure of the stimulus. By using smoke, we established that the plume structure was similar to that of plumes in larger wind tunnels. The wind speed was 0.5 m/s, and the temperature was 20 °C. When a preparation was ready for investigation, it was placed 10 cm from the wind tunnel's downwind opening. The physiological type of the contacted sensillum was established by a short stimulation with 1 μg of each of the three pheromone components in succession with a 10-s pause between stimulations. The septum containing the lowest dose of pheromone (0.3 μg) was then inserted into the wind tunnel 70 cm upwind of the antenna and placed at an earlier established suitable position in the center. The responses from the preparation were recorded during 30 s. After the stimulation the preparation was allowed to recover during 30 s of clean air, whereafter the rubber septum containing the next higher concentration was introduced into the wind tunnel and allowed to stimulate the sensillum. When all four concentrations had been investigated, either a second sensillum was cut and stimulated, or a new antennal preparation was prepared. No more than two sensilla were tested on each preparation. During the experiments, all three physiological sensillum trichodeum types present on the male *A. segetum* antenna were investigated: 33 of the Z5-10:OAc-responsive type, 7 of the Z7-12:OAc-responsive type and 2 of the Z9-14:OAc-responsive type

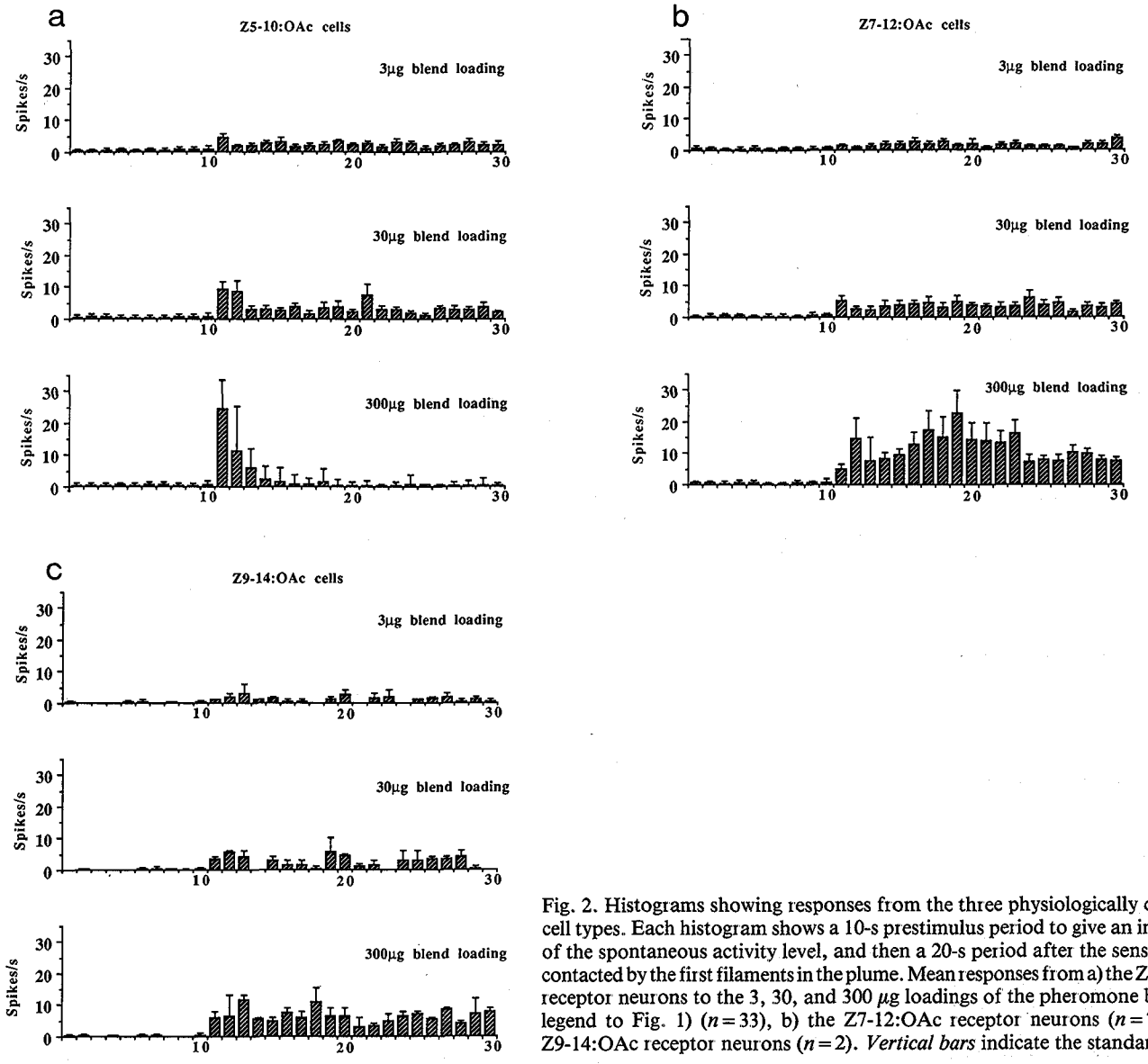

Fig. 2. Histograms showing responses from the three physiologically different cell types. Each histogram shows a 10-s prestimulus period to give an impression of the spontaneous activity level, and then a 20-s period after the sensillum was contacted by the first filaments in the plume. Mean responses from a) the Z5-10:OAc receptor neurons to the 3, 30, and 300 µg loadings of the pheromone blend (see legend to Fig. 1) ($n=33$), b) the Z7-12:OAc receptor neurons ($n=7$), c) the Z9-14:OAc receptor neurons ($n=2$). *Vertical bars* indicate the standard error

The Z5-10:OAc receptor neurons adapted very quickly when challenged with a wind tunnel plume containing a behaviorally excessive concentration (300 µg source) of the pheromone component blend (Figs. 1a, 2a). Out of the 33 investigated Z5-10:OAc receptor neurons, 31 adapted quickly when challenged with a plume from the 300 µg source. At the lower concentration stimulations (3 and 30 µg) the Z5-10:OAc receptor neurons showed the typical plume response with short bursts of action potentials, without any adaptation (Fig. 2a). In the 0.3-µg plume the bursts were very weak and rare. The response of Z7-12:OAc and Z9-14:OAc receptor neurons were similar to those of the Z5-10:OAc cells to the intermediate flight-evoking septum loadings (Fig. 2b,c). However, in response to even the plume from the 300-µg loading, these two cell types generally did not exhibit any adaptation at all (Figs. 1b,c, 2b,c). Out of the seven investigated Z7-12:OAc cells, only one adapted to the high-concentration plume, although the rate was somewhat slower than a typical Z5-10:OAc cell. Because of the low frequency of occurrence only two Z9-14:OAc cells were recorded from, but neither of these adapted to any of the concentrations tested.

The physiologically different pheromone receptor neurons in *A. segetum* clearly exhibit different adaptation rates to behaviorally excessive blend concentrations. The implications of this for the male moths flying upwind to pheromone at normal concentrations or becoming arrested at excessive concentrations are several. If only the adapting Z5-10:OAc sensilla are considered, it is clear how the male moth, flying upwind in an excessively concentrated pheromone plume, must experience an apparent decrease in overall concentra-

tion, since the majority of the action potentials descending from the antenna are generated by the Z5-10:OAc cells, which at the 300-μg blend loading first fire at high rates and then cease [8]. If the whole array of sensilla on the male antenna is taken into account, a second cause of arrestment can be hypothesized. Pheromone component blend ratios are often critical for the attraction of males (e.g., [2, 10]), and *A. segetum* is no exception. Baits lacking Z5-10:OAc are totally ineffective in attracting males of the Swedish *A. segetum* strain [5], even though Z7-12:OAc and Z9-14:OAc are present at the correct ratio and concentration. The central nervous system is fed information about the blend ratio of components in an *A. segetum* pheromone plume from three different sources, the three physiologically different cell types. Over 3000 sensilla on male *A. segetum* antennae have been sampled in our laboratory over the years, and only these three types of pheromone component-specific cells have been encountered, always present in separate sensilla. Presumably, blend ratios in moths are judged by CNS interneurons, or ensembles of interneurons, whose output depends on the relative strength of inputs impinging on them from the different types of receptor neurons [11]. If one of the receptor neuron types suddenly stops firing, the ratio will become unbalanced. In the present experiment the Z5-10:OAc receptor neuron quickly ceased firing when challenged with an excessively concentrated plume, whereas the receptor neurons for Z7-12:OAc and Z9-14:OAc continued sending normal levels of action potentials to the CNS. Males became arrested to this same treatment after having begun upwind flight [3], and this could be due to a perceived sudden imbalance in the blend ratio due to the differential adaptation of just the Z5-10:OAc cell, when in fact none truly exists [8].

The MGC in insects is the portion of the deutocerebrum that specifically receives input from pheromone-sensitive antennal neurons in males [13]. Boeckh et al. [12] showed a possible functional subpartitioning of the macroglomerular complex (MGC) in cockroaches. Recent evidence indicates a similar subpartitioning in male moth macroglomeruli [14]. If similar subpartitioning also occurs in *A. segetum,* then this means that whole subdivisions of the MGC of this species could be deprived of their input when the peripheral receptor neurons adapt. The output from pulse-enhancing interneurons, if they exist in *A. segetum* as they do in the antennal lobes of male *Manduca sexta* and *Heliothis virescens* [15], should also be severely compromised and the moths' ability to react to the ups and downs in concentration due to the plume's fine, filamentous structure may possibly be eliminated. It must also be taken into consideration that due to amplification of the signal, the interneurons are considerably more sensitive than the receptor neurons, and may cease to fire at concentrations that would not adapt the receptor neurons [16].

Differences between receptor neurons tuned to the same pheromone component have been carefully studied in the cabbage looper, *Trichoplusia ni* [17]. In this species it has been clearly shown that both high-sensitivity and low-sensitivity forms of receptor neurons for the main pheromone component exist. A morphological distinction between the two forms has also been demonstrated. In the *T. ni* case it is clear that the high-sensitivity receptor neuron type becomes adapted at lower concentrations than does the low-sensitivity type. More recently, however, it has been discovered that the low-sensitivity and low-spontaneous-activity (LS) form of receptor neuron is actually tuned very sensitively to a minor component. This discovery thus explains the LS receptor neurons' low affinity to the major component. In our present study and in a previous sampling [8] we have encountered a small percentage of cells that do not exhibit the same adaptation rate as most of the rest of their type do. In the Z5-10:OAc sample in this study, 2 receptor neuron cells out of 33 did not adapt, whereas 6 out of 7 Z7-12:OAc receptor neurons did not adapt. Adding our previous data to this set reveals that a total of only 4 out of 65 Z5-10:OAc cells failed to adapt, whereas 13 out of 19 Z7-12:OAc cells and 2 out of 2 Z9-14:OAc cells did not adapt. We do not yet know whether the nonadapting Z5-10:OAc cells possess fewer surface pores and are therefore less sensitive to this compound as was found for the LS cells in *T. ni* to this species' major component.

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