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Response of Single Antennal Neurons of Female Cabbage Loopers to Behaviorally Active Attractants

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The response to biologically significant odor signals in insects is dependent on a well-developed olfactory system. For most moths, successful mate location depends ultimately on a coupling between female-emitted sex pheromone components and male antennal receptor cells [1]. The reception and processing of pheromones by male moth sensory pathways have been better elucidated than host-plant odor reception by female insects. Among the possible reasons for this discrepancy is the fact that female behavioral responses have not been intensively investigated, and there appear to be many possible active combinations of components from these complex mixtures [2, 3]. A better understanding of the relationship between host-plant odors and their effects on the peripheral olfactory receptors of females is necessary inasmuch as volatile chemicals emitted by plants are often crucial in enabling female insects to locate suitable oviposition sites or food sources [4]. Adult cabbage looper moths, *Trichoplusia ni* (Hübner), locate flowers, presumably to obtain nectar, by flying upwind to floral odors [5, 6]. The first evidence that male and female antennal receptor cells of *T. ni* could be excited by floral scents was provided by the electroantennogram (EAG) [7]. However, the principal attractants within the flower odor were not identified. Cabbage looper adults that feed at flowers of the ornamental shrub, *Abelia grandiflora* (André), have been shown to respond by flying upwind to a blend of four volatiles emitted by the flowers, among which phenylacetaldehyde and 2-phenylethanol are of primary importance [5]. Female *T. ni* also have been shown to fly upwind in response to components of the male *T. ni* hairpencil pheromone [8], which includes *d*-linalool [9].

The main objective of the present study was to investigate the responsiveness of female *T. ni* single olfactory receptor cells to behaviorally identified floral attractants: phenylacetaldehyde, 2-phenylethanol, benzaldehyde, and benzyl alcohol [5]. As part of our neurophysiological survey, we also examined female single-cell responses to a component of the male hairpencil pheromone, *d*-linalool [9] and to a 50:50 mixture of the two optical isomers, *d*-and *l*-linalool (referred to as ± linalool), since we felt it was important to determine whether the behavioral responses to flower volatiles and hairpencil pheromone involved different sensory pathways. Lastly, the sensitivity of antennal neurons of female *T. ni* to their own major sex pheromone component, (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac)[10], and to puffs of the corresponding alcohol, (*Z*)-7-dodecenol (*Z*7-12:OH), a known behavioral antagonist to upwind flight in males [11], was examined.

We stimulated antennal receptor cells with 30-ms puffs from glass cartridges loaded with an odorant-bearing strip of filter paper. For each of the compounds, 10-, 30-, 100-, 300-, and 1000-µg cartridges were prepared by pipetting 10 µl of diluted hexane solutions onto filter-paper strips. Prior to recordings, the effective concentration of each chemical emitted during a 30-ms puff from a cartridge was quantified in order to make more accurate comparisons among receptor cell responses to compounds presented at equivalent airborne concentrations (Table 1). Briefly, the procedure involved trapping puffs of a compound on glass wool onto which a known amount of internal standard was added. The glass wool was stripped with solvent, which was then concentrated with N_2 before being injected into the gas chromatograph for quantification.

Recordings from antennal neurons of 1- to 5-d-old female *T. ni* were obtained by

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Table 1. Effective concentrations (ng ±SD) of various compounds of known behavioral importance to female *T. ni* that are emitted during a puff from a glass cartridge loaded with different dosages of each chemical

Compound	n	(µg (in 10 µl hexane solution) loaded on filter paper in a glass cartridge 10	30	100	300	1000
*Z*7-12:AC	3	12.9 ± 2.9	26.9 ± 11.3	31.9 ± 17.4	63.6 ± 49.6	72.4 ± 30.7
*Z*7-12:OH	3	4.7 ± 0.3	5.1 ± 1.0	25.1 ± 5.9	42.9 ± 3.4	43.6 ± 23.7
Benzaldehyde	3	6.0 ± 0.9	55.8 ± 10.8	286.7 ± 60.7	675.3 ± 53.8	5105.9 ± 284.0
Phenylacetaldehyde	3	12.8 ± 6.0	17.6 ± 4.2	589.5 ± 76.5	621.4 ± 41.4	2925.4 ± 1676.5
2-Phenylethanol	3	4.7 ± 2.4	27.2 ± 11.3	239.5 ± 123.0	787.1 ± 132.2	2419.7 ± 480.5
Benzylalcohol	3	45.5 ± 8.5	69.9 ± 6.9	547.0 ± 217.8	2026.8 ± 1144.0	2013.6 ± 724.4
d-Linalool	3	48.4 ± 14.4	614.9 ± 95.3	2094.4 ± 398.6	5988.6 ± 1108.6	4401.6 ± 1013.9
±-Linalool	3	64.4 ± 12.7	328.3 ± 57.5	3794.5 ± 137.9	9524.5 ± 1894.1	9382.7 ± 1950.7

the cut-sensillum technique [12, 13], which was also used to investigate male *T. ni* receptor cell responses to components of the female-emitted sex pheromone [14]. We connected successfully with 168 trichoid sensilla; however, in only 49 of those connections did we observe spiking activity by receptor cells to any of the test compounds. In 7 of the 49 sensilla, a receptor cell was excited by a single floral odorant, usually phenylacetaldehyde or 2-phenylethanol (Fig. 1A, B). In one sensillum, at least two receptor cells seemed to be present, based on spike amplitudes, with a smaller spiking cell responding to phenylacetaldehyde and a larger spiking cell responding to benzaldehyde (Fig. 1C). In all of these sensilla, the responses to any of the floral compounds were highly phasic. Antennal receptors in female *T. ni* sensilla that were tuned to a single floral odorant did not exhibit a response to puffs from cartridges below the 1000-µg filter-paper loading. Our collection technique indicated that 1000-µg flower-odorant cartridges released between 2000 and 5000 ng of odorant per puff, depending on which of the four flower odorants was puffed (Table 1). When the cells within these same sensilla were exposed to a comparable emitted dosage of *d*-linalool or ± linalool released from 100-µg cartridges (Table 1), no action potentials were generated (Fig. 2). Thus, some of the sensilla on female *T. ni* antennae contain flower-odorant-specific cells, and these cells may be tuned to a single floral compound. Puffs from cartridges containing the female sex pheromone component, *Z*7-12:Ac, or the alcohol *Z*7-12:OH could not be com-

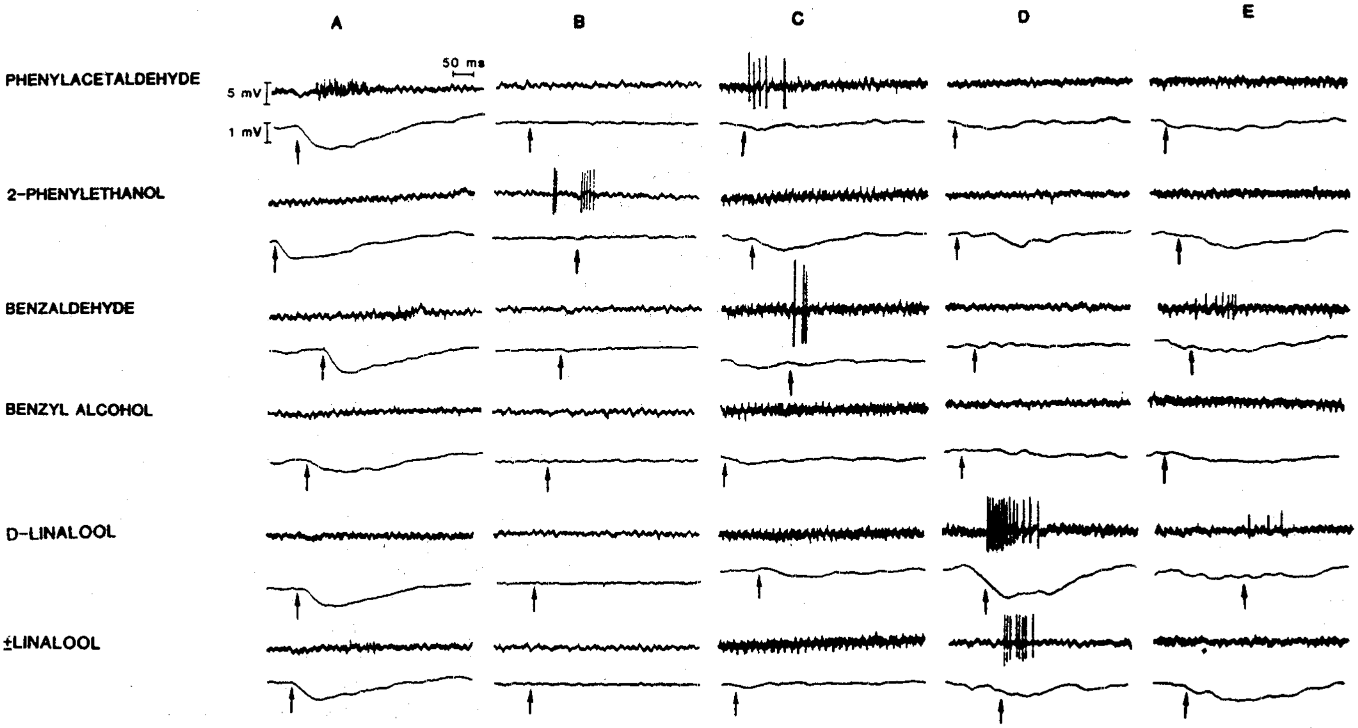

Fig. 1. Typical AC (upper) and DC (lower) responses of female *T. ni* antennal receptor cells to behaviorally active attractants identified from flowers (phenylacetaldehyde, 2-phenylethanol, benzaldehyde, and benzyl alcohol) and o male hairpencil pheromone component (*d*-linalool). A 50:50 mixture of *d*- and *l*-linalool (± linalool) also was tested. *Arrows* represent stimulus presentation. Columns A-E represent single-cell responses from cells within five separate sensilla

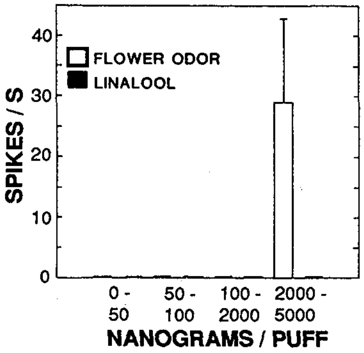

Fig. 2. Spiking activity (mean ± SD) elicited from single antennal neurons of female *T. ni* when stimulated with quantified amounts of flower odorant (phenylacetaldehyde, 2-phenylethanol, benzaldehyde, benzyl alcohol) or male hairpencil pheromone (*d*-linalool, ± linalool) released during a puff from a filter-paper-loaded glass cartridge. Histogram bars for flower odorant represent the activity of cells responding to any of the tested flower volatiles, as responses to each compound were similar. Action potentials were not evoked by comparable emitted dosages of male hairpencil pheromone (*d*-linalool or ± linalool)

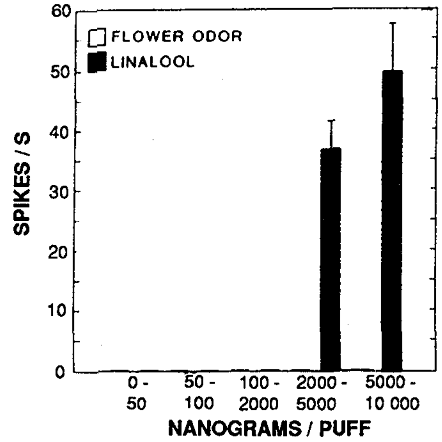

Fig. 3. Spiking activity (mean ± SD) elicited from single antennal neurons of female *T. ni* when stimulated with quantified amounts of male hairpencil odorant. Histogram bars represent the activity of cells that responded to either *d*-linalool alone or to ± linalool, as responses were similar. Action potentials were not evoked by comparable emitted dosages of any of the flower odorants

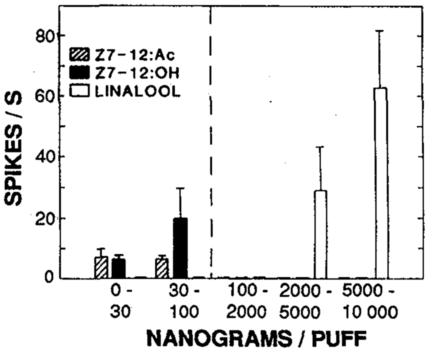

Fig. 4. Spiking activity (mean ± SD) elicited from single antennal neurons of female *T. ni* when stimulated with quantified amounts of the female-emitted sex pheromone, *Z*7-12:Ac, and the corresponding alcohol, *Z*7-12:OH, and to a male hairpencil pheromone component (linalool). These sensilla did not contain cells that responded to puffs from any of the flower-odorant cartridges. The vertical, dashed line indicates that even 1 000-μg cartridges of *Z*7-12:Ac and *Z*7-12:OH did not release more than 73 ng of odorant per puff, while 10-μg cartridges of linalool did not release less than 40 ng of odorant per puff (see Table 1)

pared to puffs from flower-odorant or hairpencil-pheromone cartridges because even 1 000-μg cartridges of *Z*7-12:Ac and *Z*7-12:OH released less than less than >2.4 ng of odorant per puff (Table 1).

In addition to the flower-odorant-specific cells, we also recorded responses from 24 sensilla that contained cells tuned specifically to *d*-linalool (Fig. 1 D). The threshold level for action potentials occurred at the 100-μg loading (Table 1), and there was no significant increase in firing when these cells were exposed to puffs from 1 000-μg cartridges (Fig. 3). Puffs from flower-odorant cartridges with similar release rates (Table 1) did not stimulate these cells. The addition of *l*-linalool to *d*-linalool did not accentuate or seem to hinder the generation of spikes when compared to *d*-linalool alone (Fig. 1 D); therefore, we have combined the responses to *d*-linalool and ± linalool to get an overall impression of the effect of linalool on spiking activity (Fig. 3). In 12 of the 49 recordings, receptor cells were stimulated by puffs from 100-μg cartridges of linalool and also by puffs from 1000-μg cartridges of a flower odorant, most commonly benzaldehyde (Fig. 1 E). The 100-μg linalool cartridges and 1 000-μg flower-odorant cartridges produced comparable emission rates (Table 1).

Antennal receptor cells of male *T. ni* are very sensitive to the major and minor components of the female sex pheromone [14–16]. Evidence provided by EAG recordings has also suggested that the antennae of female *T. ni* possess sensilla with receptor neurons sensitive to their own major pheromone component, *Z*7-12:Ac [17, 18]. We located five female sensilla harboring a receptor cell that responded to *Z*7-12:Ac, and we conclude from our single-cell recordings (Fig. 4) that these cells have a low *Z*7-12:Ac threshold, similar to that of male antennal receptors for this compound [15]. Receptor cells began responding to puffs of *Z*7-12:Ac from 100-μg cartridges; action potential frequency was not significantly increased in response to puffs from 1 000-μg cartridges (Fig. 4), even though these cartridges emitted twice as much pheromone (Table 1). In male *T. ni*, sensilla that contained a cell responsive to *Z*7-12:Ac nearly always contained a second cell that was tuned to *Z*7-12:OH [14]. Similar cells appear to be located in female sensilla (Fig. 4). Spiking activity was evoked by stimulation with puffs of *Z*7-12:OH from both the 100- and 1 000-μg cartridges. A cell occurred within all of these same sensilla that could also be stimulated by linalool (Fig. 4), but at much higher concentrations, i.e., when cartridges released at least 2 000 ng per puff (100- and 1 000-μg filter-paper loadings) (Table 1).

Our data show that most of the receptor cells that respond to *A. grandiflora* floral compounds respond to only a single compound (Figs. 1 A, B). For instance, in the seven sensilla that housed a cell that responded to a single flower odorant, that cell was stimulated by phenylacetaldehyde in four of the sensilla, by 2-phenylethanol in two of the sensilla, and by benzaldehyde in one of the sensilla. These types of cells seem to be at odds with the idea that host-plant volatiles are detected by "generalist" receptor cells in insect antennae. However, more recordings from sensilla containing such specialized cells are needed to verify this conclusion. The lack of response to any of the compounds by 119 out of 168 of the cells is intriguing and may also be indicative of further specificity to as yet untested compounds from other classes of odorants.

Our electrophysiological data correspond nicely with the emission rate and behavioral data concerning flower hostfinding by *T. ni* [5, 6]. In our study, spiking activity was evoked by receptor cells that responded to a floral compound only when puffs emitted ≥ 2000 ng of odorant (Fig. 2), which is within an order of magnitude of the amounts emitted by some flowers. A single *A. grandflora* flower emits 4000 ng of phenylacetaldehyde per 24 h and 2600 ng of 2-phenylethanol [5]. The period of maximal emission for *A. grandiflora* may actually be of much shorter duration. In addition, during the period of maximum release, each flower of the night-blooming jessamine, *Cestrum nocturnum* L., a shrub visited by cabbage loopers, releases ca. 800 ng of phenylacetaldehyde per 0.5 h and 2600 ng of benzaldehyde [6]. The existence of receptor cells specifically tuned to either phenylacetaldehyde or 2-phenylethanol is interesting in that wind-tunnel investigations showed that either of these two compounds alone was capable of stimulating upwind flight and source contact by males [5] or females (phenylacetaldehyde only) [6].

Although the emission rates for the flower odorant that evoked action potentias in female receptor cells were not too dissimilar to the rates that can evoke upwind flight in females [5, 6], the rate of linalool emission that was needed in order to generate spiking by female cells was considerably higher than the behaviorally relevant concentrations found in male hairpencil pheromone [9]. Either more is emitted than is found in the hairpencil pheromone, or these linalool-sensitive cells in hairs may be present in order to perceive plant-emitted linalool [19] released at higher rates than male-emitted linalool. There may also be receptors in other types of sensilla, e.g., basiconic, which have a lower threshold for this pheromone component.

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