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Neurons discovered in male *Helicoverpa zea* antennae that correlate with pheromone-mediated attraction and interspecific antagonism

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Abstract Responses of single receptor neurons in the antennae of male *Helicoverpa zea* to sex pheromone components and to behavioral antagonists were recorded using a cut-sensillum extracellular recording technique. Three types of sensilla were identified from sampling 325 male-specific sensilla trichodea located at the lateral edge of antennomeres. The majority of these sensilla (71%) contained a receptor neuron tuned to the principal sex pheromone component (*Z*)-11-hexadecenal. A second sensillar type (10%) contained a receptor neuron that responded only to (*Z*)-9-tetradecenal. A third sensillar type (19%) contained a large-spiking neuron tuned to the secondary pheromone component (*Z*)-9-hexadecenal, but this neuron also could be stimulated to equivalent spike frequencies by the same emitted amounts of (*Z*)-9-tetradecenal. A smaller-spiking neuron in this sensillar type responded to two compounds known to act only as behavioral antagonists, (*Z*)-11-hexadecen-1-ol and (*Z*)-11-hexadecenyl acetate, and to (*Z*)-9-tetradecenal. Cross-adaptation studies confirmed the presence of one large- and one small-spiking neuron in the third sensillar type. Dose-response studies correlated to collected stimuli amounts showed that the large-spiking neuron in the third sensillar type was equally tuned to (*Z*)-9-hexadecenal and (*Z*)-9-tetradecenal, whereas the smaller-spiking neuron was far more sensitive to (*Z*)-11-hexadecen-1-ol and to (*Z*)-11-hexadecenyl acetate than to (*Z*)-9-tetradecenal.

Key words *Helicoverpa zea* · Noctuidae · Lepidoptera · Single-cell recordings · Antennal neurons

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Abbreviations *Z11-16:Ald* (*Z*)-11-hexadecenal · *Z9-16:Ald* (*Z*)-9-hexadecenal · *Z9-14:Ald* (*Z*)-9-tetradecenal · *Z11-16:OH* (*Z*)-11-hexadecen-1-ol · *Z11-16:Ac* (*Z*)-11-hexadecenyl acetate

Introduction

In North America, four sympatric heliothine species share (*Z*)-11-hexadecenal (Z11-16:Ald) as the principal component in their sex pheromone blends but have different secondary components. The secondary component in the pheromone blend of *Helicoverpa zea* is (*Z*)-9-hexadecenal (Z9-16:Ald) (Klun et al. 1980a; Pope et al. 1984; Vetter and Baker 1984); in *Heliothis virescens*, (*Z*)-9-tetradecenal (Z9-14:Ald) (Roelofs et al. 1974; Tumlinson et al. 1975; Klun et al. 1980b; Vetter and Baker 1983; Teal et al. 1986); in *Heliothis phloxiphaga*, (*Z*)-11-hexadecen-1-ol (Z11-16:OH) (Raina et al. 1986); and in *Heliothis subflexa*, (*Z*)-11-hexadecenyl acetate (Z11-16:Ac) and Z11-16:OH (Teal et al. 1981; Klun et al. 1982; Heath et al. 1990). In addition, pheromone emissions or gland extracts in all four species indicate the presence of a second common constituent, Z9-16:Ald, but with the exception of *H. zea* the function of this compound could not be determined in behavioral bioassays. Field studies demonstrated that adding Z9-14:Ald, the secondary sex pheromone component of *H. virescens*, to the two-component *H. zea* blend caused reduction in trap catch (Shaver et al. 1982). Also, placing *H. virescens* and *H. zea* females in the same trap resulted in significantly reduced capture of *H. zea* males (Haile et al. 1973; Roach 1975; Carpenter et al. 1984; Lopez and Witz 1988). Likewise, the addition of Z11-16:OH, a sex pheromone component of *H. phloxiphaga*, to the *H. zea* blend decreased male attraction (Shaver et al. 1982; Teal et al. 1984). The antagonistic behavioral response of male *H. zea* to blends containing Z9-14:Ald might be explained by the discovery of receptor neurons in the antennae of male *H. zea* that are tuned to Z9-14:Ald (Almaas et al. 1991; Christensen et al. 1990, 1991).

These neurons were thought, therefore, to play a key role in the arrestment response of male *H. zea* to the *H. virescens* pheromone blend.

Similar behavioral and neurophysiological data exist for male *H. virescens* and Z11-16:Ac. Wind-tunnel studies have shown that Z11-16:Ac mediates antagonism of upwind flight in male *H. virescens* (Vickers and Baker 1997), and neurophysiological studies have demonstrated the existence of receptor neurons (Berg et al. 1995) as well as antennal lobe projection neurons (Christensen et al. 1995) that are tuned to this antagonist.

The presence of receptor neurons in heliothine species tuned to pheromone-like compounds that are not part of their own pheromone could be an indicator of behavioral antagonism that would ensure that males orient only to conspecific females. Detailed knowledge about antennal receptor neuron specificity and sensitivity to sex pheromone components and behavioral antagonists would provide a firm foundation for understanding intra- and interspecific chemical communication of heliothine species, and will benefit research on their neural pathways, resulting in a better understanding of the neurobiological basis of mate-seeking behaviors.

The aim of this study was to examine the response characteristics of individual antennal receptor neurons in male *H. zea* to interspecific compounds and to screen the antennae for the existence of receptor neurons that are tuned to the second *H. zea* sex pheromone component Z9-16:Ald. In addition, by constructing tuning curves for these neurons we wanted to perform more accurate dose-response analyses than had previously been done, by quantifying the amounts of compounds actually issuing from the stimulus cartridges.

Materials and methods

Insects

H. zea larvae were reared on a modified pinto-bean diet (Shorey and Hale 1965). Following pupation the moths were separated according to sex and placed in separate environmental chambers on a 14:10 h L:D cycle. Emerging adults were supplied with a 10% sugar solution, and all the males used in the experiments were between 1 and 4 days old.

Chemical stimuli

Serial dilutions of Z11-16:Ald, Z9-16:Ald, Z9-14:Ald, Z11-16:OH, and Z11-16:Ac were obtained from neat material stored in our laboratory. The purity of the compounds was >98% as determined by capillary gas chromatography-mass spectrometry (GC-MS), and the compounds were free of cross-contaminations. All GC-MS analyses were performed by using a Hewlett-Packard 5890 GC with a direct interface to a Hewlett-Packard 5972 mass selective detector (30-m DB-225 capillary column, electron impact, 70 eV). Serial dilutions of the compounds were made in redistilled HPLC-grade hexane, and the solutions were stored in 4-ml glass vials at −20 °C.

Single-cell recordings and stimulation

To record from the olfactory receptor neurons within individual antennal sensilla, a cut-sensillum technique developed by Kaissling (1974) was used. Briefly, an antenna of a moth was excised from the head, and the antennal base was placed in a Beadle-Ephrussi Ringer (Ephrussi and Beadle 1936) saline-filled micropipette and grounded with a Ag/AgCl wire. The antenna was maneuvered with a micromanipulator until a single sensillum trichodeum rested on the sharpened blade of a stationary, vertically positioned glass knife, with its tip hanging over the edge. The sensillum tip was cut off using a mobile glass knife (Van der Pers and Den Otter 1978) placed in a second micromanipulator, and the cut end was contacted with a second saline-filled micropipette containing a Ag/AgCl recording electrode. Sensilla were selected close to the dorsal (scale-covered) surface on the proximal end of the middle of the antenna, known to house the sexually dimorphic sensilla trichodea (Grant et al. 1989; Almaas et al. 1991). The antenna was continuously bathed in a stream of purified, humidified air (10 ml s^{-1}) that passed through a 12-cm-long glass tube (7 mm ID) whose outlet was positioned 1 cm from the antenna. Prior to exposure to any of the test compounds, the spontaneous activities of the receptor neurons within each sensillum were monitored over a 30-s period.

For each of the compounds tested, 10 µl of a diluted solution was pipetted onto a filter-paper strip held in a Pasteur pipette (15 cm long), hereafter referred to as the odor cartridge. Stimulus doses tested were 3, 10, 30, 100, and 300 µg. Solutions were checked by GC-MS to confirm that the amounts of respective compounds at a particular concentration were equal. Receptor neurons were exposed to uniform 20-ms puffs of odor-bearing air by injecting a 0.3-ml puff through the tip of a stimulus cartridge placed into a hole in the glass tube 10 cm from the outlet, using a pulsed stimulus flow controller (Syntech, Hilversum, The Netherlands). Stimulus compounds were selected in random order, beginning with the 3-µg odor cartridges and working upward to the 300-µg odor cartridges. Every puffed stimulus was preceded and followed by a puff with a solvent blank cartridge. Preliminary dose-response recordings showed that no spiking activity could be observed after 2 s post-stimulation at the highest stimulus dose. Therefore, the time period that elapsed between every puff was set at 10–30 s. At the end of each dose-response test, the antenna was stimulated with an additional 100-µg dose of either Z11-16:Ald or Z9-14:Ald to verify the initial neuronal activities. Response data obtained from neurons that failed to respond a second time to test stimulus were not included in the final dose-response analysis. The data were collected from no more than five sensilla per antenna.

The preamplified electrical responses of the receptor neurons (a.c. and d.c.) were monitored on a Philips PM3335 digital storage oscilloscope. They were then recorded and stored onto video tapes by using a Vetter model 420F four-channel FM recorder for later data analysis. Acquisition and spike analyses were performed using a PC-AT compatible microcomputer equipped with an analog to digital conversion board (Das-16, Keithley Metrabyte) running SAPID software (Smith et al. 1990). Capture of the amplified (× 1000) and filtered (100–10 000 Hz) analog signal was triggered by a 5-V signal generated by the flow controller at the onset of the stimulus pulse. The captured signal was digitized at a rate of 15 000 samples/s for 1 s and saved in a data file. Data files were further processed using an automatic template procedure to extract number of spikes, duration, and mean action potential amplitudes.

Cross-adaptation studies

Preliminary dose-response recordings indicated that one physiologically identifiable sensillar type consistently contained a large-spiking neuron tuned to Z9-16:Ald, whereas Z9-14:Ald consistently stimulated both the large- and a smaller-spiking neuron in this sensillum. Both Z11-16:OH and Z11-16:Ac also stimulated the small-spiking neuron in this sensillum. Therefore, cross-adaptation studies were conducted to determine if the different spiking activity could be attributed to the same neuron or to different neurons that produced similar-sized spikes (Kaissling et al. 1989). Using a stimulus flow controller and single-cell recording techniques previously described, two sets of experiments were performed, using

two different stimulus onset and offset regimes. In the first experiment, sensilla containing both large- and smaller-spiking neurons (identified by a single 100-ms puff of Z9-16:Ald at a dose of 30 μg) were stimulated for 0.1 s with an interstimulus interval (the time between the end of the first stimulus and the beginning of the second stimulus) of 0.4 s. The stimulus regime consisted of the following four stimulus pairs: (1) Z9-16:Ald/Z9-16:Ald, (2) Z9-14:Ald/Z9-14:Ald, (3) Z9-16:Ald/Z9-14:Ald, and (4) Z9-14:Ald/Z9-16:Ald. The stimulus dose tested was 30 μg. For the second experiment, the interstimulus interval was set at 0.2 s and the stimulus regime consisted of the following nine stimulus pairs: (1) Z9-14:Ald/Z9-14:Ald, (2) Z11-16:OH/Z11-16:OH, (3) Z11-16:Ac/Z11-16:Ac, (4) Z9-14:Ald/Z11-16:OH, (5) Z11-16:OH/Z9-14: Ald, (6) Z9-14:Ald/Z11-16:Ac, (7) Z11-16:Ac/Z9-14:Ald, (8) Z11-16:OH/Z11-16:Ac, and (9) Z11-16:Ac/Z11-16:OH. Stimulus doses were 30 μg (Z9-14:Ald) and 100 μg (Z11-16:OH and Z11-16:Ac). Stimulus doses were selected based on similar spike frequencies in dose-response tests. No full adaptation of the neurons was attempted with only a second stimulus. A relative decreased spike frequency was attempted by stimulating with a second stimulus. Stimulus pairs were presented randomly, and recorded and digitized spike train data were analyzed using the previously described SAPID procedure.

Stimulus collections

Compounds emitted from odor cartridges, as in the dose-response stimulation experiments, were collected as they issued from the pipette tip in 25-cm-long glass collection tubes (3 mm ID). The tip of an odor cartridge (aged overnight at room temperature), containing a filter-paper strip (3 cm × 0.5 cm, Whatman no. 1) loaded with 10 μl of a serially diluted stimulus, was inserted into a collection tube, and the connection was sealed with Teflon tape. The collection tube was placed in a container (20 cm long × 3 cm ID) filled with dry ice. The odor cartridge was then connected to the flow controller, and the released compound of the odor cartridge was collected using 20-ms pulses with an air flow set at 15 ml s^{-1}. Collection tubes were washed with 30 μl of HPLC-grade hexane containing (*Z*)-10-pentadecenyl acetate (50 pg μl^{-1}) as an internal standard. Collected amounts were analyzed by using GC-MS in selective ion mode. Collected amounts were calculated as mean (3 replicates/dose) picograms/pulse (0.3 ml) and corrected for differences in relative abundance of the selected ions relative to the internal standard. Trap breakthrough was checked and confirmed negative for all odor cartridges at the highest dose level by analyzing collected material in a second, in-series-connected, glass tube.

Collection protocol was as follows: Z11-16:Ald, Z9-16:Ald, and two doses of Z11-16:Ac (30 μg and 100 μg), 3072 pulses; Z11-16:OH and two doses of Z11-16:Ac (3 μg and 10 μg), 4096 pulses. Collections for Z9-14:Ald doses were performed by using 80-cm-long glass tubes washed with 90 μl of the internal standard (50 pg μl^{-1}), using 128 pulses for each dose. Collections of Z11-16:OH were analyzed using a 30-m DB-1 capillary column. All other collections were analyzed by using a 30-m DB-225 capillary column. All collection tubes were stored at −20 °C prior to analyses by GC-MS.

Results

Single-cell recordings and stimulation

Single-cell recordings were obtained from 325 sensilla using 84 antennae. Recordings from these trichoid sensilla showed three different response profiles. Neurons in 71% of these sensilla (231 out of 325) responded only when stimulated by Z11-16:Ald (Fig. 1, type A). Neurons in 33 sensilla (10%) responded only when stimulated by Z9-14:Ald at any of the dosages tested (Fig. 1, type B). The third sensillar type (19%) contained at least two neurons: a large-spiking neuron responded when stimulated with Z9-16:Ald and Z9-14:Ald, and a smaller-spiking neuron responded when stimulated with Z11-16:OH, Z11-16:Ac, and Z9-14:Ald (Fig. 1, type C). In all of the sensilla, relatively low (1–3 spikes/s) background firing by one or two receptor neurons was observed prior to stimulus presentation. None of these neurons responded to the hexane controls above the background firing level.

The dose responses of the Z11-16:Ald-specific neurons showed an increase in firing rate in a dose-related manner, reaching an optimal firing rate when stimulated with 55.5 pg/puff (Fig. 2). Dose-response profiles for the Z9-14:Ald-specific neurons were similar to those for Z11-16:Ald but occurred at one order of magnitude higher dose with an overall lower spike frequency (Fig. 2). In the C-type sensilla, the two compounds, Z9-16:Ald and Z9-14:Ald, that stimulated a large-spiking neuron showed similar spike frequencies (Fig. 2). Similar spike frequencies were recorded for the smaller-spiking neurons when stimulated with Z9-14:Ald, Z11-16:Ac, and Z11-16:OH, but the spike frequencies elicited by Z9-14:Ald occurred at 10–100 times higher doses compared with the spike frequencies elicited by Z11-16:Ac and Z11-16:OH (Fig. 2).

The spike frequencies of the large-spiking neuron in response to Z9-14:Ald were significantly higher than those of the smaller-spiking neuron, except at the 30-μg loadings ($P > 0.05$, Student's *t*-test).

The dose-response relationship for the neurons present in the three types of sensilla varied with age. Three- to 4-day-old males showed a significant decrease in average sensitivity at every dose and for every stimulus, but the response profiles were similar to that of 1- to 2-day-old males apart from a higher responses variability (data not shown).

Almost all of the sampled (98.6%) sensilla in the dose-response tests responded to puffs of test stimuli at the end of the dose-response series and the antennal preparations were viable for at least 1 h.

Cross-adaptation studies

The single-cell responses of the large-spiking neuron located within the C-type sensilla showed that this neuron could be partially adapted by exposure to the Z9-16:Ald/Z9-16:Ald pair, with a 0.4-s interstimulus interval (Fig. 3). Similarly, larger-spiking neurons and smaller-spiking neurons were partially adapted when stimulated with the Z9-14:Ald/Z9-14:Ald pair. Exposing the large-spiking neurons to the Z9-16:Ald/Z9-14:Ald or to the Z9-14:Ald/Z9-16:Ald pairs resulted in a similar pattern of responses to those evoked by the homologous pairs.

In the cross-adaptation study with the small-spiking neurons (Fig. 4), the responses of small-spiking neurons

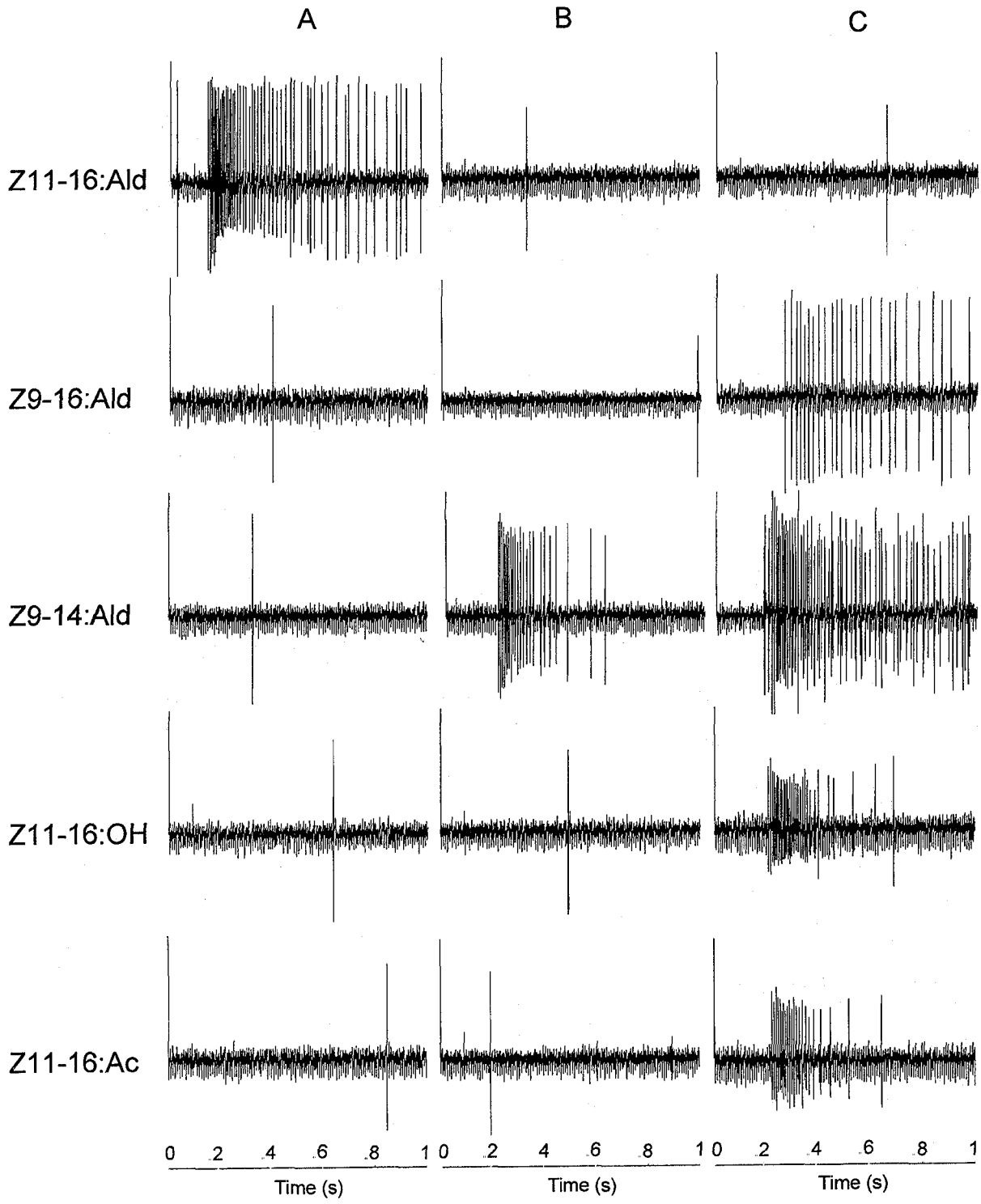

Fig. 1A–C Typical electrophysiological responses of neurons within three types of trichoid sensilla in the antennae of male *H. zea*. Stimuli were the two sex pheromone components of this species [30 μg of (*Z*)-11-hexadecenal (Z11-16:Ald) and 30 μg of (*Z*)-9-hexadecenal (Z9-16:Ald)], and three behavioral antagonists [30 μg of (*Z*)-9-tetradecenal (Z9-14:Ald), 100 μg of (*Z*)-11-hexadecenyl acetate (Z11-16:Ac), and 100 μg of (*Z*)-11-hexadecen-1-ol (Z11-16:OH)]. *Vertical line* at beginning of each tracing indicates stimulus presentation

located within the C-type sensilla were partially adapted when stimulated with three same-component pairs of Z9-14:Ald, Z11-16:Ac, and Z11-16:OH by using a 0.2-s interstimulus interval (Fig. 4, top row). At an increased interstimulus interval of 0.4 s, the small-spiking neurons became markedly more disadapted (data not shown). Stimulating the small-spiking neurons with all six stimulus combination pairs resulted in spike frequency pat-

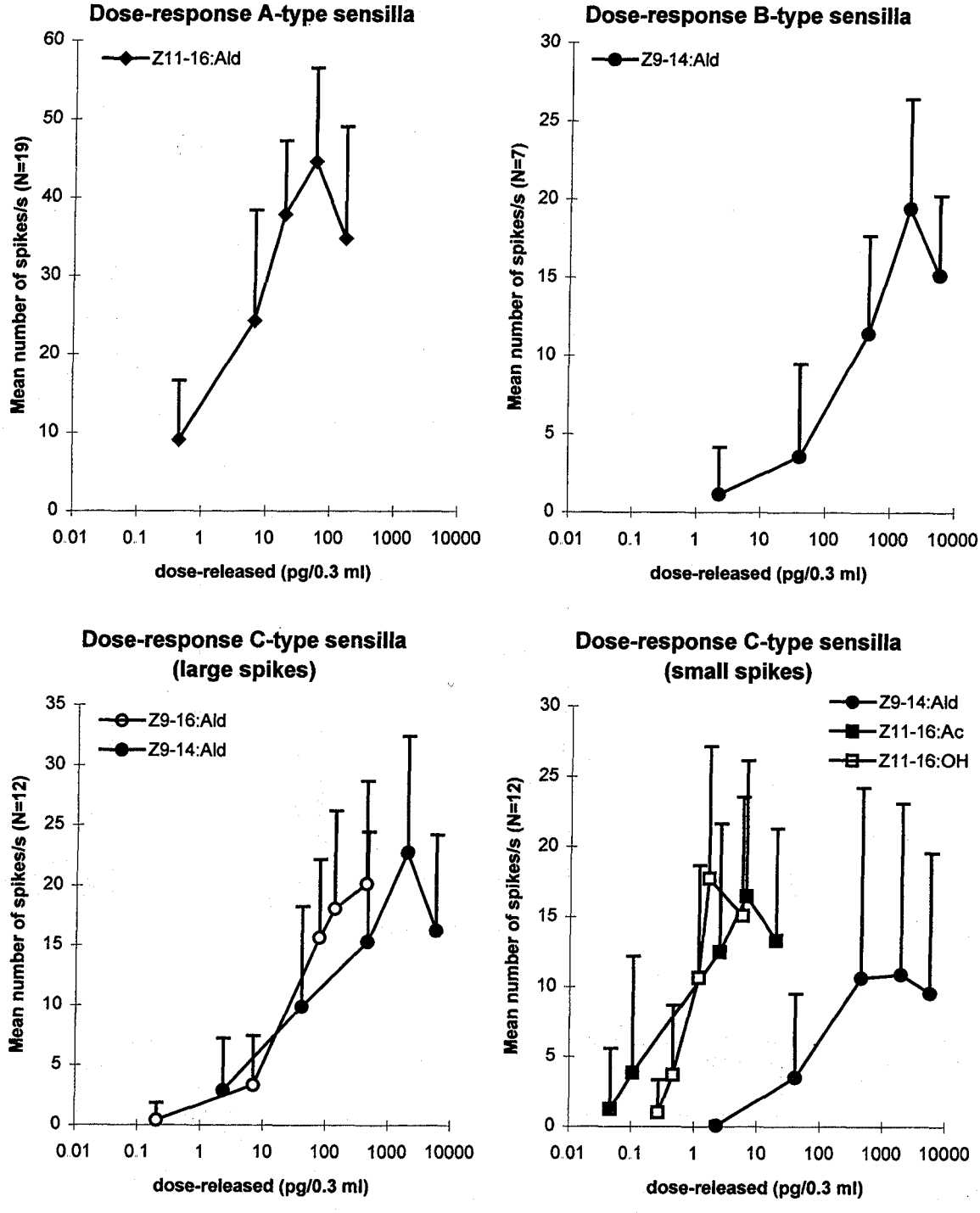

Fig. 2 Mean (±SD) dose-response relationship of the spiking activity of Z11-16:Ald-specific neurons present in A-type sensilla, Z9-14:Ald-specific neurons in B-type sensilla, and large- and small-spiking neurons in C-type sensilla. All sensilla types were presented puffs from cartridges loaded with graded amounts of the two sex pheromone components and the three behavioral antagonists. Spike frequencies are plotted against the amounts of compound released from filter paper (see Table 1). Z9-16:Ald stimulated a large-spiking neurons in C-type sensilla, Z11-16:OH and Z11-16:Ac stimulated a smaller-spiking neuron, and Z9-14:Ald stimulated both the large- and smaller-spiking neuron

terns similar to those of the same-component pairs (Fig. 4, middle and bottom rows).

Stimulus collections

The collection data from odor cartridges are presented in Table 1. The highest amounts collected were from odor cartridges loaded with Z9-14:Ald. On average, loadings of Z9-14:Ald released 5.8–14.3 times more compound than similar loadings of Z9-16:Ald. Dosages of Z11-16:Ald and Z9-16:Ald had similar release rates at 3 μg and 10 μg, but at the higher loadings, Z9-16:Ald-cartridges emitted 4.1–2.4 times higher amounts of compound than similar loadings of Z11-16:Ald. The

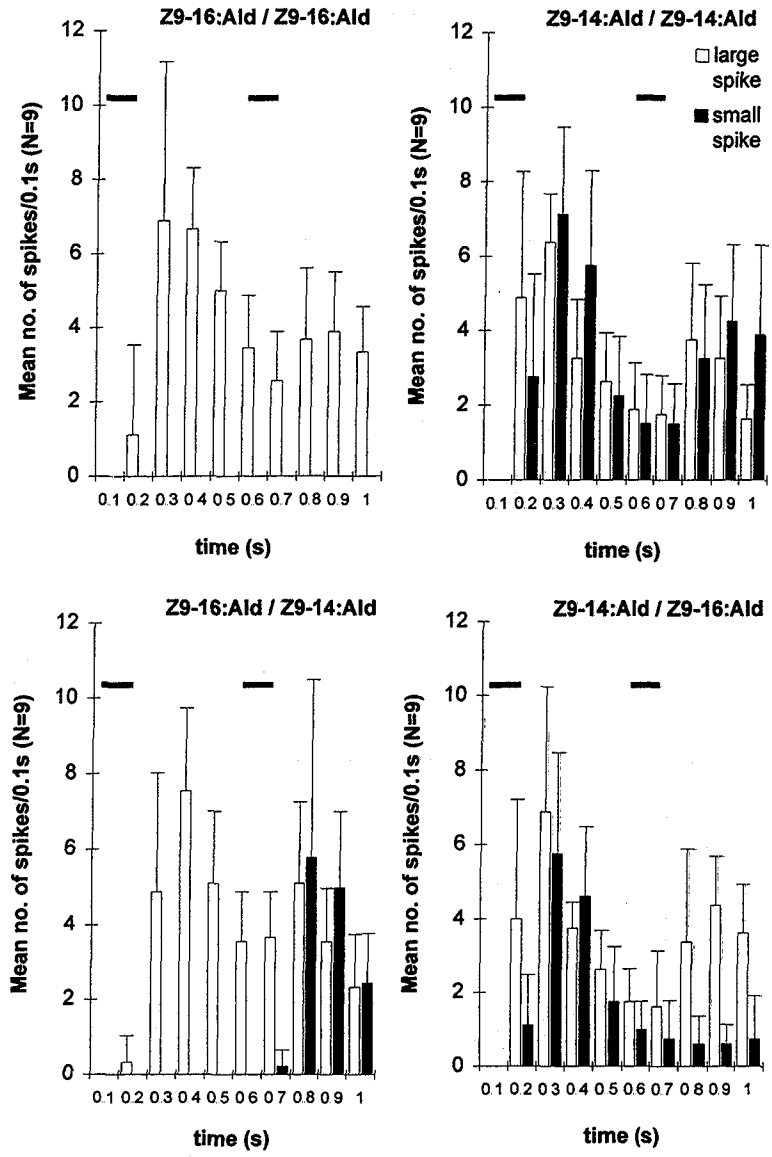

Fig. 3 Histograms showing the spike frequencies (mean ± SD) of large- and small-spiking neurons present in C-type sensilla in response to four stimulus pairs. Each histogram shows two 100-ms stimulations (*black horizontal bars*) with an interstimulus interval of 0.4 s. Stimulus dose was 30 μg

lowest emission rates occurred from cartridges loaded with either Z11-16:Ac or Z11-16:OH.

Discussion

We propose that our results now explain the neuronal basis for the peculiar ability of Z9-14:Ald to act both as an antagonist and an agonist to upwind flight by *H. zea* males depending upon whether it is blended with Z11-16:Ald at high or low ratios, or solely as an antagonist when it is added even in small ratios to the two pheromone aldehydes (Shaver et al. 1982; Vickers et al. 1991). The explanation lies in our discovery of a type of sensillum (C-type) that contains a large-spiking neuron that is tuned equally to Z9-16:Ald and Z9-14:Ald. Given that any neuron that is optimally stimulated by Z9-16:Ald should be involved in agonistic, upwind flight behavior, this neuron then explains how Z9-14:Ald can substitute for the absence of Z9-16:Ald (Vickers et al. 1991) in eliciting attraction of *H. zea* males. This neuron also is consistent with the existence of antennal lobe interneurons that exhibit a similar ability to respond to both Z9-16:Ald and Z9-14:Ald (Christensen et al. 1991).

Antagonism in response to the addition of Z9-14:Ald, on the other hand (Shaver et al. 1982; Vickers et al. 1991), is explained by the second (small-spiking) neuron we discovered that responds to Z9-14:Ald. This receptor neuron is likewise housed in the C-type sensillum, but we found that it is yet more sensitive to Z11-16:Ac and Z11-16:OH, two known antagonists to attraction to the pheromone blend (Fadamiro and Baker, in press; Vetter

▶

Fig. 4 Histograms showing the spike frequencies (mean ± SD) of large- and small-spiking neurons present in C-type sensilla in response to nine stimulus pairs. Each histogram shows two 100-ms stimulations (*black horizontal bars*) with an interstimulus interval of 0.2 s. Stimulus dose was 30 μg (Z9-14:Ald) and 100 μg (Z11-16:OH and Z11-16:Ac)

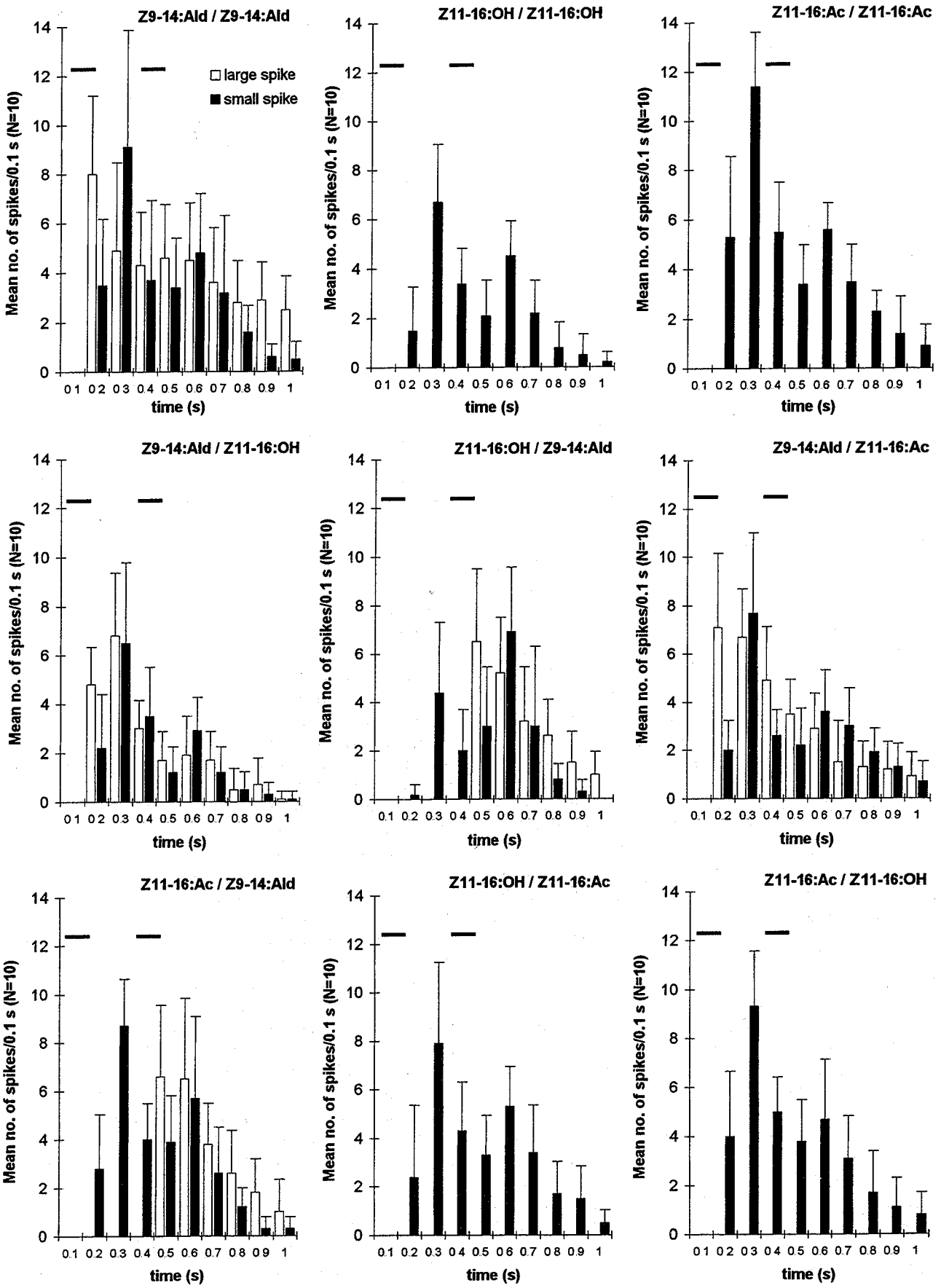
Z9-14:Ald / Z9-14:Ald
Z11-16:OH / Z11-16:OH
Z11-16:Ac / Z11-16:Ac
Z9-14:Ald / Z11-16:OH
Z11-16:OH / Z9-14:Ald
Z9-14:Ald / Z11-16:Ac
Z11-16:Ac / Z9-14:Ald
Z11-16:OH / Z11-16:Ac
Z11-16:Ac / Z11-16:OH
□ large spike
■ small spike
Mean no. of spikes/0.1 s (N=10)
time (s)
0 2 4 6 8 10 12 14
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

Table 1 Mean (±SD) amounts of emitted compounds collected per 0.3-ml air pulse (15 ml s^{-1}) from odor cartridges containing filter papers loaded with different doses of neurophysiological stimuli

Dose loaded (μg)[a]		Picograms/0.3 ml									
		Z11-16:Ald		Z9-16:Ald		Z9-14:Ald		Z11-16:Ac		Z11-16:OH	
	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
3	3	0.46	0.15	0.20	0.07	2.33	1.71	0.05	0.03	0.27	0.00
10	3	6.54	2.13	6.99	3.38	40.78	6.83	0.11	0.05	0.46	0.00
30	3	18.67	2.63	77.60	14.07	457.74	55.31	2.50	1.08	1.15	0.13
100	3	55.47	10.26	135.79	40.34	1940.49	166.87	6.38	2.21	1.65	0.13
300		168.24		435.39		5673.30		19.47		5.58	

[a] Collected amounts for 300-μg doses were extrapolated

and Baker 1983; Raina at al. 1989). Because this neuron is tuned to two known antagonists and also can be stimulated by Z9-14:Ald, such stimulation should be involved in antagonistic behavioral responses such as arrestment of upwind flight. The large-spiking (agonistic) neuron responds with a higher spike frequency to Z9-14:Ald than does the smaller-spiking (antagonistic) neuron, explaining how a small amount of Z9-14:Ald can mimic positive behavioral responses that occur to Z9-16:Ald (Vickers et al. 1991). However, at higher doses the increase in spike frequency of the small-spiking antagonistic neuron will counteract any agonistic behavior that might occur in conjunction with the excitation of the large-spiking neuron.

Almaas et al. (1991), using tungsten electrodes, reported that neurons in 10% (4 out of 36 total recordings) of the *H. zea* male-specific sensilla trichodea responded to Z9-14:Ald and that these neurons were secondarily responsive to Z9-16:Ald. Considering the smaller sample size in the Almaas et al. (1991) study and our ability in discriminating the various receptor neurons by spike size and our measurements of release rates, we suggest that Almaas et al. (1991) were recording from two different neurons, not one, both sensitive to Z9-14:Ald and with one of the two being responsive to Z9-16:Ald. They also declared that the (single) neuron that they found in these four recordings was secondarily responsive to Z11-16:Ac and Z11-16:OH, but following our results it is clear that only the small-spiking neuron is sensitive to these compounds.

Thus, we now can understand that the responsiveness to Z9-16:Ald and Z9-14:Ald by one type of neuron in the C-type sensillum is involved in agonistic behavior and the responsiveness to Z11-16:Ac, Z11-16:OH, and Z9-14:Ald by a different neuron is likely involved in antagonistic behavioral responses. It is very difficult to add enough Z9-16:Ald to Z11-16:Ald to result in reduction of upwind flight by male *H. zea* (Vickers and Baker 1991), and this is mirrored in our receptor neuron recordings, because Z9-16:Ald did not, at any tested dosage, cause the antagonistic small-spiking neuron to fire.

The assignment of tuning sensitivities, neuron identities, and sensillar homes for the neurons that we discovered are supported by our use of the cut-sensillum technique, quantification of emission rates from pipettes, and cross-adaptation results. For instance, at first glance, the dose-response study based on filter-paper loadings indicated that the large-spiking neuron in the C-type sensillum had a lower threshold to Z9-14:Ald (see Fig. 5). However, on average, the range of release rates from the Z9-14:Ald cartridges turned out to be 5.8–14.3 times higher when compared with the same dosage range on the filter papers of the Z9-16:Ald cartridges, and based on these results we concluded that the large-spiking neuron is equally sensitive to the sex pheromone component Z9-16:Ald and Z9-14:Ald. A similar analysis was made for the small-spiking neuron found in the same sensilla.

The relative emission rates of stimuli from filter paper-glass cartridges generally follows what one would predict based on the different chain lengths as well as functional groups that are correlated with vapor pressure. Because this is the first study that has actually captured and then measured picogram amounts of pheromone stimuli issuing from filter-paper-in-glass odor cartridges, it is difficult to say whether other, seemingly anomalous values are actually due to measurement errors or are real. For instance, the slight differences in emission rates of Z9-16:Ald and Z11-16:Ald may be due to these compounds, although having identical molecular weights, possessing different affinities for the filter paper or the glass-walled surfaces in the cartridges. Differences in the shapes and polarities of these two molecules, after all, account for their quite different behaviors on the surfaces of both polar and apolar capillary columns that allow them to be separated chromatographically. Changes in the behaviors of molecules injected onto GC columns at non-saturation (low) quantities compared with saturation quantities (exceeding column capacity) are also well-known, and may relate to the shifts in glass cartridge emission rates of these two compounds in this study as the loading rate increased. More of the kinds of measurements that we made in the present study will be needed in the future in order to find patterns in odor emission rates between and within studies from various laboratories using different systems.

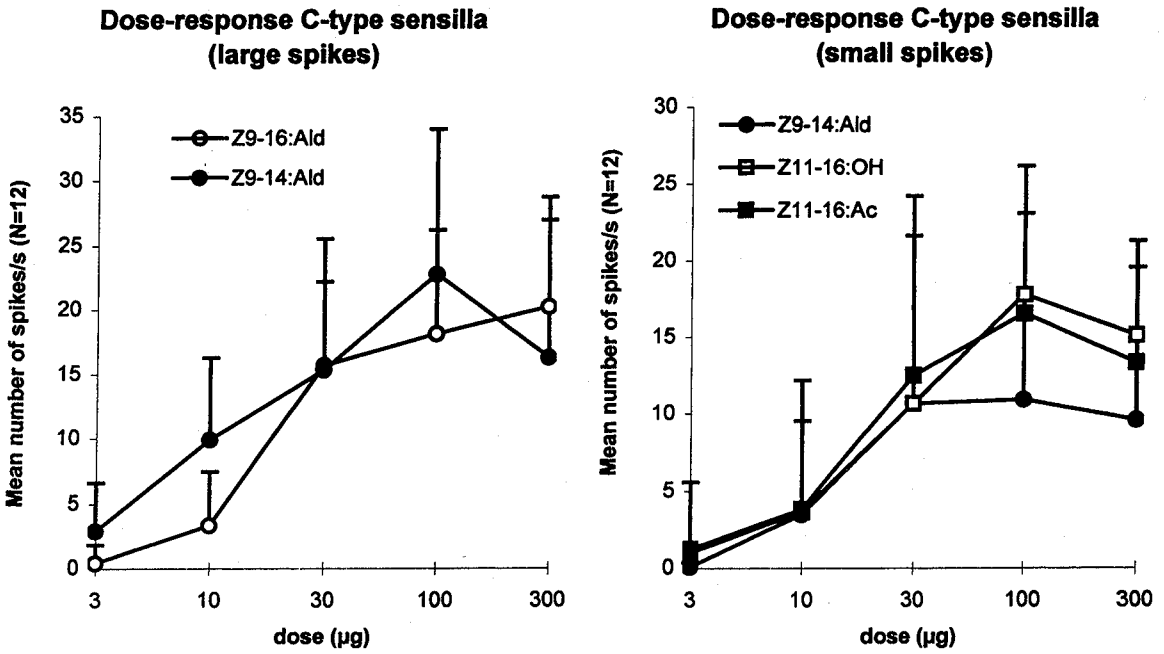

Fig. 5 Mean (±SD) dose-response relationship of the spiking activity of large- and small-spiking neurons in C-type sensilla. Sensilla were presented puffs from cartridges loaded with graded amounts of the two sex pheromone components and the three behavioral antagonists. Spike frequencies are plotted against the amounts of compound loaded on filter paper. Z9-16:Ald stimulated a large-spiking neurons in C-type sensilla, Z11-16:OH and Z11-16:Ac stimulated a smaller-spiking neuron, and Z9-14:Ald stimulated both the large- and smaller-spiking neuron

Our cross-adaptation study showed that Z9-14:Ald, Z11-16:OH, and Z11-16:Ac stimulated one and the same small-spiking neuron. Considering only the dose of compound administered to the filter paper, the dose-response study showed about an equal sensitivity of the small-spiking neuron for all three antagonists (see Fig. 5). However, the release rates issuing from Z11-16:OH- and Z11-16:Ac-stimulus cartridges were found to be at least two orders of magnitude (100–800 times) lower than those resulting from the same loadings of Z9-14:Ald. These findings support the conclusion that the small-spiking neuron is tuned to Z11-16:OH and Z11-16:Ac and secondarily receptive to Z9-14:Ald.

The Almaas et al. (1991) study showed that about 90% of the neurons recorded from the male-specific sensilla trichodea were selectively responsive to Z11-16:Ald, and that the remaining 10% responded optimally to Z9-14:Ald. Like Almaas et al. (1991), we found that the majority (71%) of neurons in the sensilla we sampled contained a neuron tuned to Z11-16:Ald. However, in addition, we discovered a new neuron housed in a separate (B-type) sensillum, that responded exclusively to Z9-14:Ald regardless of dosage. Almaas et al. (1991) did not report such a neuron. The behavioral effects mediated by this neuron's activity remain to be determined, and it will be interesting to see whether these sensilla exist to detect the presence of an interspecific compound (Z9-14:Ald) in the plume, or whether these sensilla are present possibly to house an agonistic neuron that is a vestige of when Z9-14:Ald may have been a *H. zea* pheromone component (Vickers et al. 1991).

Field studies have shown the antagonistic effects of *H. virescens* female-emitted Z9-14:Ald to *H. zea* males (Shaver et al. 1982). Wind tunnel studies, similarly, have shown that both Z11-16:OH and Z11-16:Ac mediate antagonism of upwind flight in both *H. virescens* (Vetter and Baker 1983; Vickers and Baker 1997) and *H. zea* males (Raina et al. 1989; Fadamiro and Baker, in press). The small-spiking neuron in the antennae of male *H. zea* is most responsive to Z11-16:Ac and Z11-16:OH as well as to Z9-14:Ald at higher dosages. Thus, activation of this broadly tuned "antagonist" neuron could explain the arrestment response of male *H. zea* to blends containing small amounts of any of these compounds, which are emitted by females of three other North American heliothine species, *H. subflexa* (Teal et al. 1981; Klun et al. 1982; Heath et al. 1990), *H. phloxiphaga* (Raina et al. 1986), and *H. virescens* (Roelofs et al. 1974; Tumlinson et al. 1975; Klun et al. 1980b; Vetter and Baker 1983; Teal et al. 1986). Even though these three sympatric species also emit the same two 16-carbon aldehydes, Z11-16:Ald and Z9-16:Ald, used by *H. zea* in its sexual communication, this broadly tuned antagonist receptor neuron may help to ensure that *H. zea* males orient only to conspecific females. Research, currently under way, on where these newly discovered receptor neurons project to in the macroglomerular complex of the antennal lobe may further characterize the neurophysiological pathways involved in pheromone olfaction.

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