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Mixture Interactions in Moth Olfactory Physiology: Examining the Effects of Odorant Mixture, Concentration, Distal Stimulation, and Antennal Nerve Transection on Sensillar Responses

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Abstract

The insect olfactory system is challenged to decipher valid signals from among an assortment of chemical cues present in the airborne environment. In the moth, *Heliothis virescens*, males rely upon detection and discrimination of a unique blend of components in the female sex pheromone to locate mates. The effect of variable odor mixtures was used to examine physiological responses from neurons within sensilla on the moth antenna sensitive to female sex pheromone components. Increasing concentrations of heliothine sex pheromone components applied in concert with the cognate stimulus for each neuronal type resulted in mixture suppression of activity, except for one odorant combination where mixture enhancement was apparent. Olfactory receptor neuron (ORN) responses were compared between moths with intact and transected antennal nerves to determine whether specific instances of suppression might be influenced by central mechanisms. Type A sensilla showed little variation in response between transected and intact preparations; however, recordings from type B sensilla with transected antennal nerves exhibited reduced mixture suppression. Testing by parallel stimulation of distal antennal segments while recording and stimulating proximal segments dismissed the possibility of interneuronal or ephaptic effects upon sensillar responses. The results indicate that increasing concentrations of "noncognate" odorants in an odor mixture or antennal nerve transection can produce variation in the intensity and temporal dynamics of physiological recordings from *H. virescens* ORNs.

Key words: antennal nerve, *Heliothis virescens*, mixture effects, single-sensillum recording

Introduction

The coding of olfactory information requires deciphering meaningful signals from a complex odor background. Many electrophysiological studies of olfaction have focused on the detection and activity elicited in the olfactory system by stimulation with single odorants (O'Connell and Akers 1989). However, almost all relevant cues are produced, released, and received as mixtures of chemicals (Mayer and McLaughlin 1995; Witzgall et al. 2004). In the case of insects, this may range between volatile emissions from host plants and sex pheromones released by females to attract males (Cardé 1984; Bernays and Chapman 1994).

Insects exhibit robust and stereotypical behavior to relevant olfactory cues, being important primarily in mating and host selection in the Lepidoptera, as well as alarm, recognition, or aggregation in other taxa (Howse et al. 1998). For plant volatiles, the specific complement of odors produced by a host can be interpreted as agonistic or antagonistic by ovipositing females. De Moraes et al. (2001) found that *Heliothis virescens* F. females can discriminate between host plants under attack by conspecific larvae and mechanically damaged plants based on nocturnal volatile emissions from the plants in wind tunnel assays. Female *H. virescens* can discriminate between male *H. virescens* and *Heliothis subflexa* Guenée hairpencil extract (which contain a similar complement of odorants at different ratios) during courtship (Hillier and Vickers 2004). Particularly well studied are male upwind flight responses to specific blends and ratios of female sex pheromone. In addition, mixtures of odorants derived from black poplar leaves, *Populus nigra*, produced both enhancement and inhibition of wind tunnel flight and source contact when mixed with pheromone

in *Helicoverpa armigera* Hübner (Deng et al. 2004). The reception and coding of odor complexity therefore appears very important in eliciting insect behavior.

Coding of olfactory cues may occur before the first synapse within the olfactory system. Specificity of olfactory receptors on olfactory receptor neurons (ORNs) to ligands provides for a first-order filter of olfactory signals, distinguishing background "noise" from relevant information (Mustaparta 1997). In insects (and other animals), ORNs are often described as "specialist" (usually pheromone-sensitive cells) or "generalist" (usually plant odors) due to specificity in activity (Kaissling 1974). Mixture interactions between odorants have been proposed to cause blend enhancement or suppression through molecular inhibition with the receptor site, indirect effects on second messengers, or possibly through interrupting ionic conductance (Michel et al. 1991; Lucero et al. 1992; Ache 1994; Kurahashi et al. 1994; Olson and Derby 1995; Daniel et al. 1996; Sanhueza et al. 2000; Carlsson and Hansson 2002). In some cases, odorants have been shown to cause excitation in one ORN type and inhibition in another (Shields and Hildebrand 2001). In lobsters, toads, and rats, ORNs can be hyperpolarized through odor-induced potassium conductance (Michel et al. 1991; Sanhueza et al. 2000). Ochieng' et al. (2002) found that spike activity from *Helicoverpa zea* Hübner ORNs to Z11-hexadecenal (Z11-16:Ald), a primary pheromone component, was increased in the presence of Z11-16:Ald mixed with linalool, a common plant volatile. This presents the possibility that observed "mixture effects" may be due, in part, to activity enhancement or suppression of ORN activity at the sensillar level before reaching the antennal lobe (AL).

The enhancement or suppression of ORN activity in response to mixtures has been documented in crustaceans (Steullet and Derby 1997; Cromarty and Derby 1998), mammals (Oka et al. 2004), and insects (Carlsson and Hansson 2002; Ochieng' et al. 2002). In lobsters, studies have demonstrated that mixtures most often evoke a decrease in response to a cognate ligand when mixed with one or more additional odorants (Steullet and Derby 1997; Cromarty and Derby 1998). Mouse ORNs sensitive to eugenol indicated concentration-dependent antagonism by mixtures of eugenol with either methyl isoeugenol or isosafrole, as indicated by intracellular calcium response (Oka et al. 2004). Other studies, however, have also indicated mixture suppression of pheromone-sensitive ORN responses when plant odors are introduced to the pheromone mixture (den Otter et al. 1978; Van der Pers and den Otter 1978).

Few studies have investigated the effects of blends on insect neurophysiology, and of these studies, most have investigated reception and activity produced specifically by combinations of pheromone components (O'Connell et al. 1986; Akers and O'Connell 1988). O'Connell et al. (1986) found variable effects on spike frequency when minor components of the *Trichoplusia ni* Hübner female sex pheromone were presented as a blend with the major pheromone component, Z7-12:OAc. In *Spodoptera litura* F, synergy has also been observed at the peripheral level. More recently, Carlsson and Hansson (2002) found that in *Agrotis segetum* Schiff sensilla, there were only occasional instances of suppression when stimulated with mixtures of pheromone components. Electrophysiological recording from the Japanese beetle, *Popillia japonica* Newman, indicated the presence of both blend-sensitive and blend-suppressed ORNs when stimulated with mixtures of this species' female sex pheromone, (R)-japonilure, and a behavioral antagonist, (S)-japonilure (Nikonov and Leal 2002).

The effect of blends of behaviorally relevant odorants on ORN activity in *H. virescens* was investigated through single-sensillum tip recordings. Specifically, the effect of increasing concentrations of noncognate odorants on the stimulus-evoked activity of type A (sensitive to the major component of the *H. virescens* female sex pheromone: Z11-16:Ald) and type B (sensitive to a minor pheromone component: Z9-tetradecenal [Z9-14:Ald]) sensilla was examined. This strategy was used primarily to determine if odor quality effects might be present at this peripheral sensory level. In addition, ORN responses were compared between recordings from moths in which the antennal nerve was either intact or transected to determine whether peripheral interactions might be a result of anterograde feedback from the AL. Finally, in a third experiment, noncognate odorants were applied to the distal region of the antenna while recording from proximal sensilla to determine if mixture effects could be attributed to direct stimulation of the sensillum or through interneuronal interactions on the antenna.

Materials and methods

Insects

Male moths were obtained from the *H. virescens* colony at the University of Utah. Larvae were reared on a pinto bean diet (Shorey and Hale 1956), sexes separated following pupation and placed in an environmentally controlled chamber (Percival Scientific) at 25 °C and 60% relative humidity, and set on a reversed light schedule (14:10 h light:dark) before and after emergence. One- to four-day-old insects were used for experimentation.

Chemicals

Odorants were selected based on 2 factors. First, all the selected odorants have been previously demonstrated to have behavioral relevance to *H. virescens* (Roelofs et al. 1974; Teal and Tumlinson 1989; De Moraes et al. 2001; Hillier and Vickers 2004). This included components of host plant volatiles (linalool, β-caryophyllene, Z3-6:OAc and Z3-6:OH), components of heliothine female sex pheromones (Z11-16:Ald, Z9-14:Ald, Z11-hexadecenyl acetate [Z11-16:OAc], and Z11-hexadecen-1-ol [Z11-16:OH]), or as components of the male *H. virescens* hairpencil pheromone (hexadecyl acetate [16:OAc] and hexadecanol [16:OH]). In addition, these

odorants were of particular interest, as previous research has identified sensilla on the antenna and glomeruli in the AL for processing such odors in either male or female *H. virescens* (Hillier et al. 2006; Hillier and Vickers 2007).

Female sex pheromone components (Z11-16:Ald, Z9-14:Ald, Z9-16:Ald, Z11-16:OAc, and Z11-16:OH) were obtained from Bedoukian Research Inc. Host plant volatiles (linalool, β-caryophyllene, Z3-6:Oac, and Z3-6:OH) were provided by Dr Robert Raguso (Department of Biological Sciences, University of South Carolina). Male hairpencil components (16:OAc and 16:OH) were provided by Dr James Tumlinson (Department of Entomology, Pennsylvania State University). All odorant solutions were diluted as a decade series in hexane (10 ng to 1 mg), had >95% purity confirmed by gas chromatography (Shimadzu GC 17A, with a 30 m × 0.25 mm inner diameter [ID] DB-5 capillary column), and were stored at –20 °C.

Single-sensillum recording

Single-sensillum recording (SSR) was conducted using a cut sensillum technique as described previously (Kaissling 1974; Van der Pers and den Otter 1978; Hillier et al. 2006). Individual moths were restrained in a plastic pipette tip and their heads held in place using dental wax. Restrained moths were placed on a depression slide, their antenna was mounted in place using water-soluble correction fluid (Liquid Paper, Paper Mate), and a reference electrode was placed in the eye contralateral to the antenna being recorded.

Individual long trichoid sensilla selected from the ventral, proximal half of the antenna were cut using a "piezo-saw" technique (a resonating glass capillary mounted on a piezo attached to a function generator; Gödde 1989; Hillier et al. 2006). A saline-filled glass capillary Ag/AgCl electrode was placed over the cut tip for recording. Physiological recordings were filtered (HUMBUG, Quest Scientific), amplified (ER-1, Cygnus Technology), and monitored on an oscilloscope (GOS-620FG, Instek). Type A and type B sensilla both contain 2 neurons, each sensillum having a neuron of known odorant affinity (type A = Z11-16:Ald, type B = Z9-14:Ald), along with a second neuron with an unknown odorant affinity (Lee et al. 2006b; Baker 2009). No excitatory responses were recorded from these noncognate neurons in this study, and spikes from stimulated neurons were filtered and sorted by amplitude to distinguish responses from any spontaneous activity of noncognate neurons (Figure 1). Data were recorded filtered, and spike detection was performed using software programs devised by Dr Christoph Kleineidam (University of Würzburg, Germany) in Labview 6.1. All odor stimulation data were standardized by spontaneous spike frequency prior to stimulation before statistical analyses.

Stimulation and experimental procedure

Odorant cartridges were made by loading a 10-μL aliquot of stimulus solution onto a 5 × 30 mm piece of filter paper in a 1-mL plastic syringe. For the current study, binary mixtures were applied directly to a single filter paper (10 μL of the cognate stimulus + 10 μL of a noncognate stimulus). Odorant mixtures produced for each experiment were as follows—cognate odorant: Z11-16:Ald or Z9-14:Ald; versus noncognate odorant: Z11-16:Ald, Z9-14:Ald, Z11-16:OAc, Z11-16:OH, linalool, β-caryophyllene, Z3-6:OAc, Z3-6:OH, 16:Oac, and 16:OH. A continuous flow of charcoal-filtered, humidified air was provided at a flow rate of 1 L/min. A valve driver (Parker-Hannafin) was used to switch between the continuous airflow and the stimulus cartridge. Both stimulus and continuous flow lines entered a mixing chamber (50 mm long × 5 mm ID, with thin plastic straws inserted over the last 20 mm to smooth the flow exiting the chamber), the exit of which was positioned 10 mm from the insect's antenna. Odor stimulation was controlled automatically by Labview 6.1 software (National Instruments).

Stimulation was presented as a series of 3 × 200 ms puffs, at 1-s intervals, with 60 s between stimulation to prevent adaptation. Data were recorded from each sensillum for 2 s prestimulation and 1 s poststimulation, resulting in 6 s of recorded activity during odorant presentation.

Three experiments were conducted using different stimulus protocols:

Experiment 1—mixture interactions

For the first experiment, normal male *H. virescens* were assayed to determine whether specific odorants produced changes in odor-evoked activity when presented in concert with the cognate stimulus for an ORN within a given sensillum. The stimulus protocol involved presentation of the cognate odorant first (1 μg Z11-16:Ald for type A stimulation; 1 μg Z9-14:Ald for type B stimulation), followed by the odor mixture (1 μg cognate + 100 μg noncognate), and finally the noncognate odorant alone (100 μg). The order of noncognate odorants was randomized, and each combination of odorants was tested on every sensillum contacted. A hexane blank was presented as a control before and after each complement of odorants tested.

A main-effects analysis of variance (ANOVA) was used to determine significant differences in spike frequency or latency to odor-evoked spiking based on odorant mixture or stimulus number (1, 2, or 3). Means were separated using Fisher's least significant difference (LSD) test ($P < 0.05$), and statistical analyses were conducted using Statistica (StatSoft Inc., 1999).

Experiment 2—transected versus intact antennal nerves

For the second experiment, comparisons were made between the blend-evoked responses in sensilla on an antenna with either an intact or a transected antennal nerve. Antennal nerves were transected below the base of the antenna. A small triangular incision was made in the cuticle on the top of the moth's head, medial to the base of the antenna.

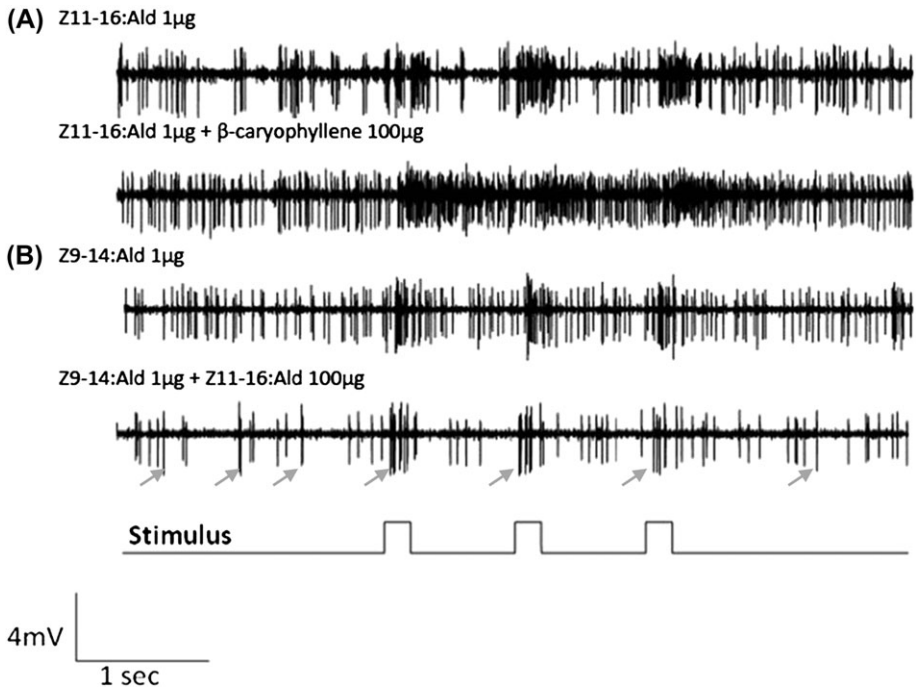

Figure 1 Sensillum ORN responses to 1 µg of a cognate ligand alone and in a mixture with 100 µg of a noncognate ligand: **(A)** ORN response (original spike trains) from a type A sensillum responding to 1 µg Z11-16:Ald alone and in a mixture with 100 µg of β-caryophyllene and **(B)** ORN response (original spike trains) from a type B sensillum responding to 1 µg Z9-14:Ald alone and in a mixture with 100 µg of Z11-16:Ald. Arrows on lower trace indicate high-amplitude spiking neurons that are stimulus driven. Six-second total recording time with a 3 × 200 ms stimulus delivery.

A thin scalpel was inserted and used to sever the antennal nerve within the head capsule. The hole was covered with vaseline to prevent hemorrhage during the experiment. Control, "intact" insects were manipulated in a similar manner; however, the antennal nerve was not cut.

This experiment also investigated the mixture effects for concentration dependence. For this experiment, the cognate odorant was presented first (1 µg "A"), followed by mixtures containing increasing concentrations of the noncognate odorant (1 µg A + 1 µg "B"; 1 µg A + 10 µg B; 1 µg A + 100 µg B), and the noncognate odorant alone (100 µg B). Order of odorant mixture presentation was randomized, and all odorant combinations were tested on each sensillum selected. Before and after each complement of odorants tested, a hexane blank was presented as a control.

A main-effects ANOVA was used with transected versus intact treatments, odorant mixture, concentration, and stimulus number as factors to evaluate either spike frequency or latency to spiking. Means were separated using Fisher's LSD test ($P < 0.05$).

Experiment 3—simultaneous proximal and distal stimulation

To further determine if mixture interactions require direct stimulation or if it might be mediated by interneuronal mechanisms within the antenna, a third experiment was designed to facilitate simultaneous odorant delivery to 2 different regions of the antenna. Odorant delivery was provided through 2 separate airstreams, one directed to the proximal base of the antenna and the other to the distal region of the antenna. Vacuum flows were set up on the opposite side of the antenna from each stimulus flow, and a Teflon barrier was set up medially across the antenna to restrict crossover of odorants between distal and proximal stimulus flows. A series of odorants were selected from the previous experiments, which caused significant changes in sensillar response. Odorant stimulation proceeded in a similar manner as Experiment 1; however, the noncognate stimuli were applied only to the distal region of the antenna, whereas SSR and stimulation with either Z11-16:Ald or Z9-14:Ald was performed only within the proximal stimulus flow.

A one-way ANOVA was used in Experiment 3 to determine if significant differences were evident between proximal stimulation with the cognate ligand or during synchronized stimulation with noncognate odorants presented distally. Means were separated using Fisher's LSD test ($P < 0.05$).

Results

SSRs were made from ORNs in type A and type B long trichoid sensilla from male *H. virescens*, and spike frequencies in response to stimulation with odorants and mixtures were recorded.

Experiment 1—mixture interactions

Forty sensilla ($N = 27$ moths) were tested for mixture interactions through stimulation with binary odors (20 each for

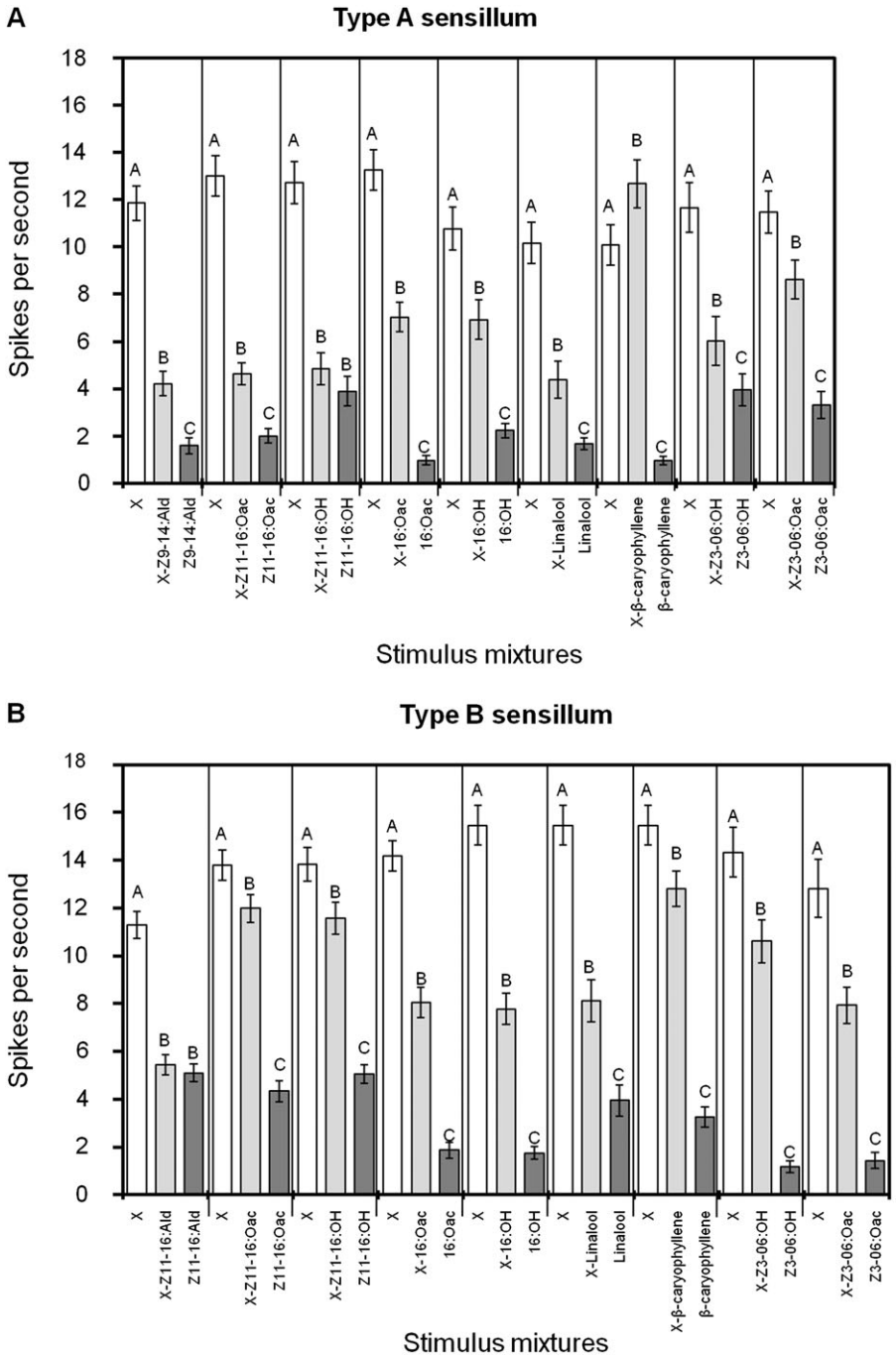

Figure 2 Spikes per second recorded from stimulation ORNs housed in **(A)** type A sensilla to 1 μg of Z11-16:Ald alone, 1 μg Z11-16:Ald mixed with 100 μg of various noncognate odorants, and 100 μg of noncognate odorants alone and **(B)** type B sensilla to 1 μg of Z9-14:Ald alone, 1 μg Z9-14:Ald mixed with 100 μg of various noncognate odorants, and 100 μg of noncognate odorants alone. Variable mixture suppression was noted in all binary mixtures excepting Z11-16:Ald + β-caryophyllene, wherein some mixture enhancement was evident. Triplets of bars (odor A = white, odor A + B = pale gray, odor B = dark gray) represented by different letters are significantly different from one another (Fisher's LSD $P < 0.05$).

type A and type B sensilla, respectively). No more than 2 sensilla were sampled from the same insect. No significant effects were noted from stimulation order (1, 2, or 3; $F_{1,228} = 1.4$, $P = 0.20$) or between repeated stimulation with the cognate ligand, Z11-16:Ald for type A ($F_{1,9} = 0.9$, $P = 0.46$) and Z9-14:Ald for type B ($F_{1,9} = 0.97$, $P = 0.46$).

Concurrent stimulation of type A sensilla with Z11-16:Ald plus a noncognate odorant typically suppressed spike

frequencies for the odorants and concentrations tested (Figures 1A and 2A). However, spike frequency was rarely reduced to the level produced by stimulation with the noncognate odorant alone (i.e., no response). The single exception to this was the mixture of 1 μg Z11-16:Ald with 100 μg β-caryophyllene, which significantly increased the mean spike frequency above that of Z11-16:Ald presented alone ($P < 0.05$). In a few instances, type A sensilla were stimulated by the noncognate odorant alone, particularly Z11-16:OH (5 cells), Z9-14:Ald (3 cells), and Z11-16:OAc (1–2 cells; Figure 2; individual data for ORNs not shown).

The 100 μg linalool + 1 μg Z11-16:Ald produced the greatest change in latency (106 ± 6.5 ms shift), whereas mixtures containing β-caryophyllene and 16:OAc had no significant effect (Table 1). Additionally, latency to stimulus-evoked spike firing in type A sensilla was significantly increased in most odor mixtures relative to 1 μg Z11-16:Ald alone (Table 2; $F_{17,1021} = 9.7$, $P < 0.001$).

Type B sensilla, which house neurons responding primarily to Z9-14:Ald, exhibited varying degrees of reduction in spike frequency when presented with any of the binary odor mixtures (Figures 1B and 2B). When combined with 100 μg Z11-16:Ald, spike frequency was reduced to level similar that of Z11-16:Ald presented alone. Reduced spike frequency due to mixture stimulation was most pronounced when the noncognate odorant was 16:OAc, 16:OH, Z3-6:OAc, or Z3-6:OH (Figure 2B). For this sensillar type, cells were also occasionally stimulated weakly by the noncognate odorant alone: Z11-16:Ald (14 cells), Z11-16:OAc (13 cells), Z11-16:OH (12 cells), and linalool (5 cells; individual ORN data not shown). In these cases, spiking frequencies were generally lower than those observed by stimulating with Z9-14:Ald alone.

Latency to stimulus-induced spiking was increased in all odor combinations presented to type B sensilla but was only significantly greater with mixtures containing 100 μg of Z11-16:Ald, 16:OAc, linalool, Z3-6:OAc, and Z3-6:OH ($F_{17,1021} = 15.3$, $P < 0.001$; Table 1). In the few instances where type B sensilla were stimulated by presentation of noncognate odorants alone, latencies were significantly greater than observed to Z9-14:Ald alone (Z11-16:Ald = +38 ± 2.1 ms; Z11-16:OAc = +44 ± 3.4 ms; Z11-16:OH = +71 ± 2.1 ms; linalool = +106 ± 5.3 ms).

Experiment 2: transected versus intact antennal nerves

Forty sensilla ($N = 40$ moths) were tested for the effects of transected and noncognate odorant concentration in blends (10 each for transected types A and B and 10 each for intact types A and B). No significant effects were noted from stimulation number ($F_{2,348} = 0.65$, $P = 0.60$) or between repeated stimulation with the cognate ligand, Z11-16:Ald for type A and Z9-14:Ald for type B ($F_{1,18} = 1.2$, $P = 0.19$).

Comparison of sensillar activity in transected and intact antennae revealed a significant reduction in the mean spontaneous spike frequency for both type A and type B sensilla (Table 2). Concentration-dependent reductions in mean spike frequencies were observed for most mixtures applied to type A sensilla (Figures 3A and 4). In a few cases, mixture responses were not significantly greater than spike frequencies observed for noncognate odorants alone (i.e., 10–100 μg 16:OH). No significant reduction was noted in blends containing Z11-16:OAc, and 10 μg β-caryophyllene produced higher spike frequencies in transected and intact treatments. Type A sensilla on antenna with a transected nerve always exhibited a reduced stimulus response to Z11-16:Ald alone,

Table 1 Mean latency in milliseconds from start of stimulation with odorant mixtures to stimulus-evoked spiking (±standard error)

	Type A			Type B		
X	1 μg Z11-16:Ald	1 μg Z11-16:Ald + 100 μg X	Mean change (ms)	1 μg Z9-14:Ald	1 μg Z9-14:Ald + 100 μg X	Mean change (ms)
Z11-16:Ald				54 (2.7)	81 (4.6)	+27 (3.8)*
Z9-14:Ald	64 (2.7)	133 (16.1)	+69 (3.5)*			
Z11-16:OAc	66 (2.4)	115 (13.0)	+49 (3.1)*	55 (2.7)	67 (3.7)	+12 (2.3)
Z11-16:OH	66 (2.6)	115 (13.9)	+49 (5.1)*	55 (2.7)	68 (4.6)	+13 (2.1)
16:OAc	75 (3.7)	81 (5.2)	+6 (0.5)	64 (3.7)	80 (5.2)	+16 (3.1)*
16:OH	76 (4.0)	97 (5.3)	+21 (2.2)*	64 (3.8)	78 (4.6)	+14 (5.0)
β-Caryophyllene	86 (5.0)	82 (4.2)	−4 (1.0)	64 (3.6)	73 (4.8)	+9 (4.2)
Linalool	74 (3.9)	180 (24.3)	+106 (6.5)*	62 (3.8)	97 (11.5)	+35 (6.5)*
Z3-06:OAc	76 (5.0)	96 (7.9)	+20 (3.3)*	58 (3.3)	86 (5.9)	+28 (4.7)*
Z3-06:OH	75 (4.8)	142 (17.7)	+66 (4.9)*	58 (3.2)	85 (5.8)	+27 (4.0)*

For each sensillar type, the first column represents latency following stimulation with the cognate stimulus only (Z11-16:Ald or Z9-14:Ald). Asterisks indicate significant differences between pairs of Z11-16:Ald or Z9-14:Ald stimulation alone or in mixtures with noncognate odorants (X). Means were separated using Fisher's LSD test ($P < 0.05$). Z3-06:OAc, Z3-hexenyl acetate; Z3-06:OH, Z3-hexen-1-ol.

and to mixtures, relative to the intact treatment (Figure 5). Otherwise, intact and transected treatments indicated similar patterns of spiking frequency in response to individual odor mixtures.

Intact type A sensillar preparations had a concentration-dependent increase in latency in response to mixtures containing Z3-6:OH (Table 3). In comparison, all odor mixtures indicated concentration-dependent increases in latency in "transected" preparations ($F_{1,1944} = 293.9$, $P < 0.001$).

Type B sensilla also exhibited a concentration-dependent reduction in spiking to odorant mixtures relative to stimulation with 1 μg of Z9-14:Ald alone (Figures 3A and 6). As noted previously, spike reduction to the mixtures was variable. For the intact treatment, lower concentrations of noncognate odorants often did not decrease spiking significantly from Z9-14:Ald alone; however, at higher dosages, reduction was always present and significant. The presence and degree of spike reduction was also variable between intact and transected treatments for type B sensilla (Figure 7). Transected treatments typically showed higher spike frequencies in response to odor mixture stimulation relative to intact treatments (despite an overall decrease in spontaneous spike frequency and unlike type A sensilla). However, Z3-6:OH showed significantly reduced responses in the transected treatment, and β-caryophyllene and Z3-6:OAc indicated no significant difference (Figure 7). Spike frequencies in response to stimulation by mixtures containing Z11-16:OAc, Z11-16:OH, 16:OH, linalool, and 16:OAc were significantly higher in transected relative to intact preparations. Increased spiking in transected treatments in response to Z9-14:Ald alone was only observed in a single case (Z9-14:Ald vs. 16:OH).

For type B sensilla, cutting the antennal nerve significantly affected latency to spiking to Z9-14:Ald in any odor mixture (Table 3; $F_{1,1939} = 13.1$, $P < 0.001$). Concentration-dependent increases in latency were noted in all odor mixtures, though this effect was weak for mixtures containing 16:OH in both transected and intact treatments and 16:OAc in the transected treatment alone.

Table 2 Spontaneous spiking activity—mean spikes per second (±standard error) recorded prior to stimulation in type A and type B sensilla from moths with intact or transected antennal nerves

	Intact	Transected	$F_{1,960}$	P
Type A	7.6 ± 0.16	4.2 ± 0.12	231.5	<0.001
Type B	6.6 ± 0.14	4.2 ± 0.16	80.7	<0.001

Means were averaged across all treatments for a given cell type.

Experiment 3: simultaneous proximal and distal stimulation

No significant differences were noted in sensillar type between spike frequency during concurrent stimulation either with distally presented noncognate odorants or with the cognate ligand alone (type A: $F_{8,161} = 0.86$, $P = 0.54$; type B:

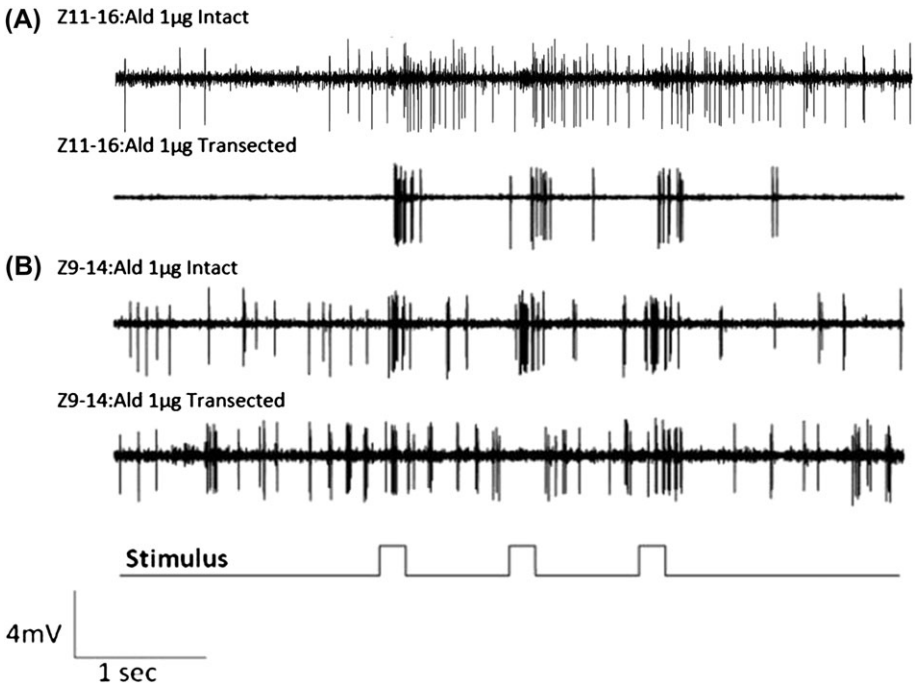

Figure 3 Sensillum ORN responses to 1 μg of a cognate ligand from intact and transected antennal nerve preparations: **(A)** ORN response (original spike trains) from a type A sensillum responding to 1 μg Z11-16:Ald on moths with intact or transected antennal nerves and **(B)** ORN response (original spike trains) from a type B sensillum responding to 1 μg Z9-14:Ald on moths with intact or transected antennal nerves. Six-second total recording time with a 3 × 200 ms stimulus delivery.

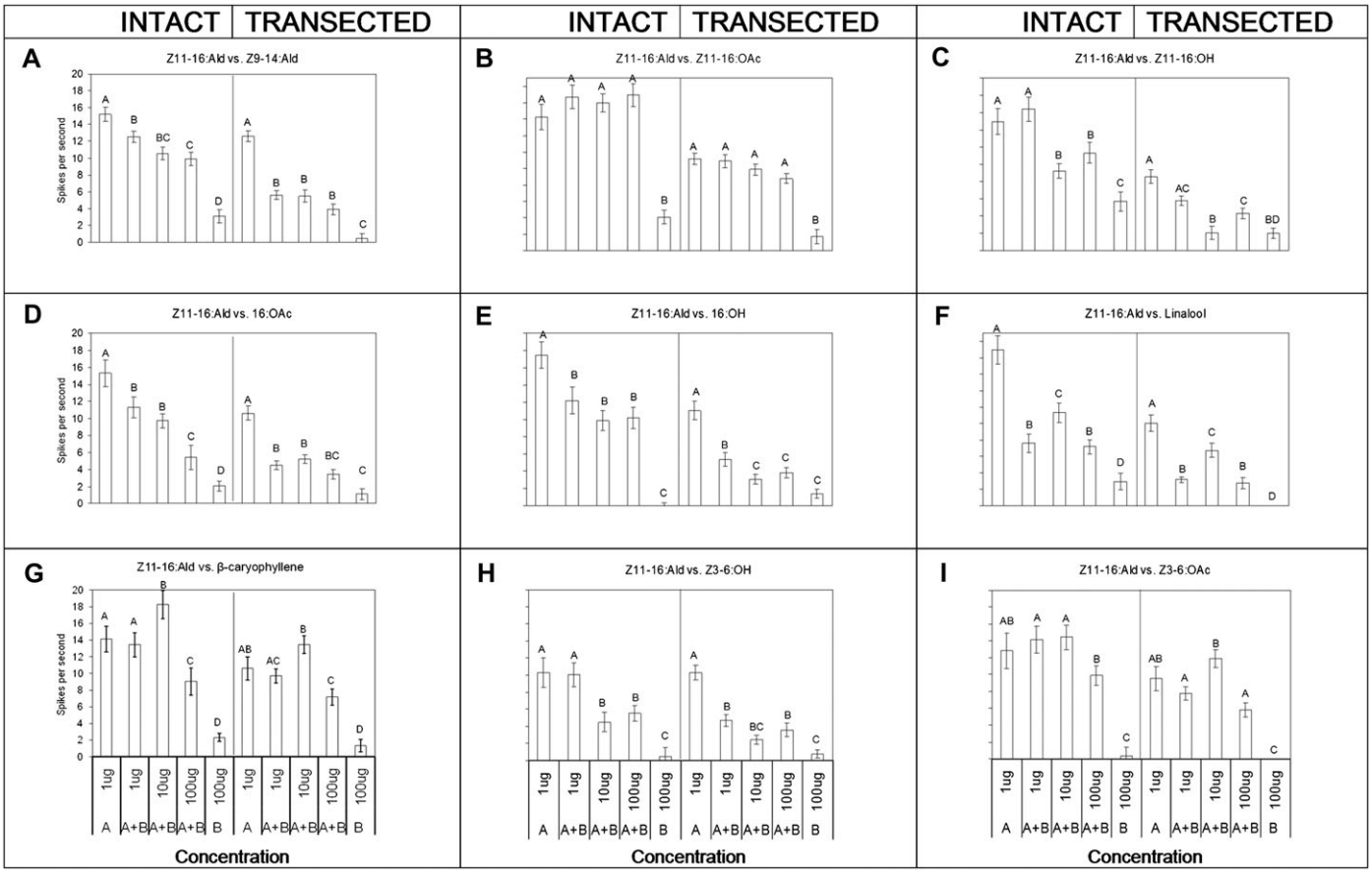

Figure 4 Type A sensillum—effects of odorant ratio in mixtures containing 1 μg Z11-16:Ald **(A)** and various noncognate odorants **(B)** on type A sensillum ORNs in preparations with intact or transected antennal nerves. In most cases, increases in the concentration of noncognate odorants mixed with Z11-16:Ald resulted in concentration-dependent decreases in ORN spiking relative to stimulation with Z11-16:Ald alone. Bars represented by different letters indicate significant differences within a treatment (intact or transected; Fishers LSD, $P < 0.05$).

$F_{8,161} = 0.45$, $P = 0.89$; Figure 8). Latency to first spiking was also not significantly different from stimulation with Z11-16:Ald or Z9-14:Ald alone (type A: $F_{8,80} = 0.75$, $P = 0.64$; type B: $F_{8,80} = 0.71$, $P = 0.68$; Table 4).

Discussion

Stimulation of *H. virescens* pheromone-specific ORNs with mixtures comprised of a cognate pheromone component and a noncognate odorant (other conspecific/heterospecific pheromone component or host plant volatile) caused modifications in spike frequency and latency to response. Most frequently, mixture interactions led to a decreased ORN firing rate compared with that produced by the cognate ligands for both types A and B sensilla. Mixture suppression attenuated neuronal responses for most odorant combinations, though the degree of suppression was variable.

Mixture interactions

When presented as a high dosage of stimulus (100 μg loading), noncognate odorants alone occasionally produced responses in both sensillar types. In many of these cases, however, molecular similarity can be inferred through chain length or functional groups present: for example, Z11-16:Ald, Z9-14:Ald, Z11-16:OAc, and Z11-16:OH. It is also of interest that all the preceding odorants are processed as agonistic and antagonistic odorants within the *H. virescens* macroglomerular complex (MGC). The MGC is a sexually dimorphic glomerular cluster within the AL of male moths, dedicated to the processing of sex pheromones. In the Heliothinae, this complex is known to contain glomeruli that process pheromone components of conspecific females and those of closely related species.

When presented as a blend, ''suppression'' was noted from each combination of female sex pheromone odorants, though mixture suppression from Z11-16:OAc and Z11-16:OH in type B ORNs was relatively weak. This was particularly curious, as these components are present in the naturally occurring pheromone mixture of many heliothine moths (Witzgall et al. 2004). In particular, Z11-16:Ald always decreased spiking in Z9-14:Ald neurons when presented as a mixture to type B sensilla. Both these odors

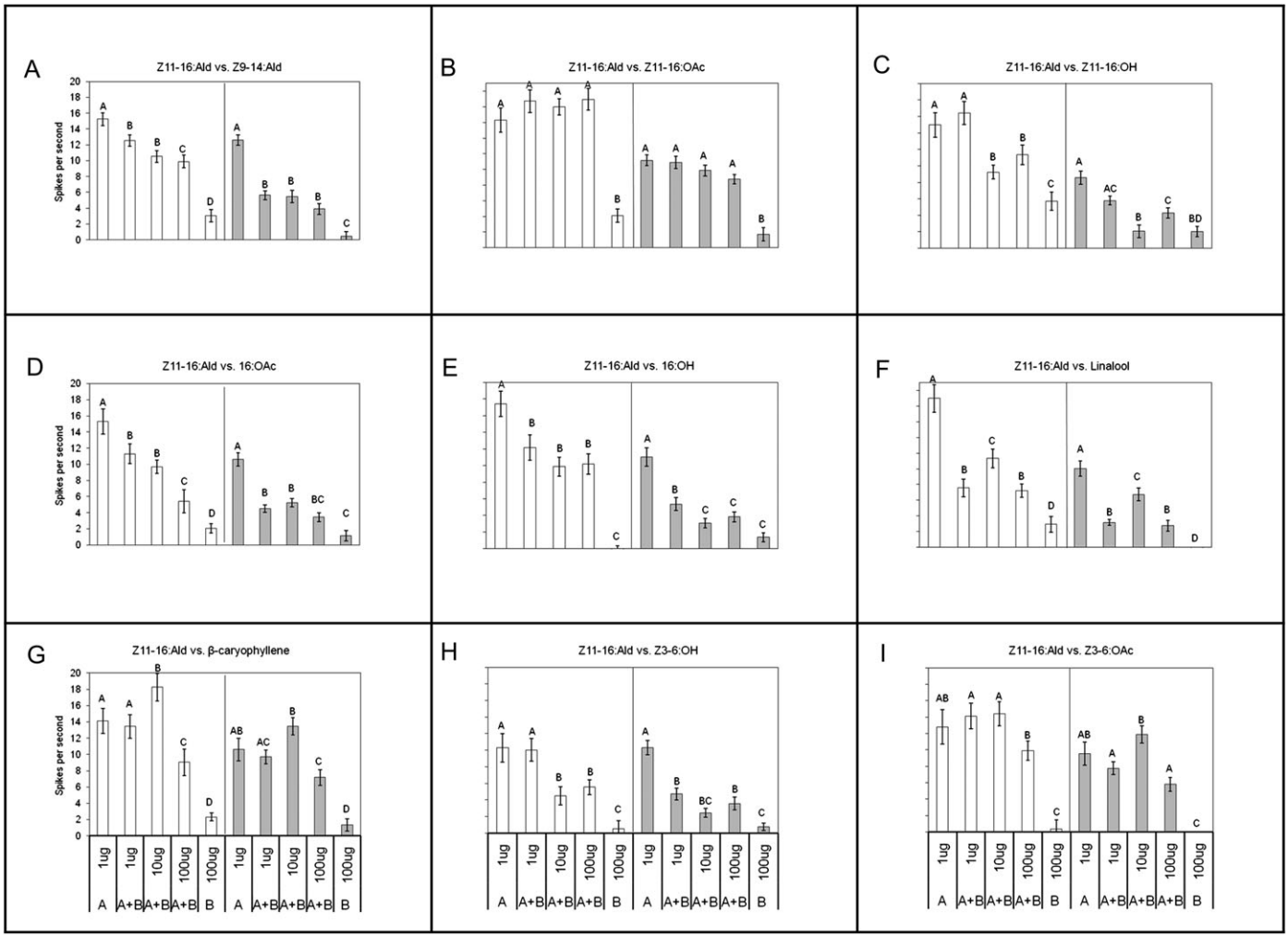

Figure 5 Type A sensillum—effects of antennal nerve transection on type A sensillum ORN responses to mixtures containing 1 μg Z11-16:Ald **(A)** and various noncognate odorants **(B)**. Overall ORN activity was decreased in transected relative to intact preparations. White = intact antennal nerve, gray = transected antennal nerve. Asterisks indicate significant differences between intact and transected pairs of mixtures; Fishers LSD, $P < 0.05$.

are essential in producing upwind flight in male *H. virescens*, so it remains unclear why one component might diminish the activity of the other on the periphery (Roelofs et al. 1974; Vetter and Baker 1983; Vickers and Baker 1997).

Carlsson and Hansson (2002) found few cases of mixture suppression in *A. segetum*. Their study, however, did not look at increasing the concentration of the noncognate stimulus above that of the cognate and instead compared binary blends at similar concentrations. In our study, mixture suppression was concentration dependent and more frequently observed at high dosages of the noncognate odorant. One possibility might be that the relatively high concentration of noncognate odorants used caused a dramatic increase in noncompetitive binding, and these molecules physically obstructed the cognate ligand from reaching the receptor site (Atema et al. 1989; Olson and Derby 1995; Kang and Caprio 1997). Nevertheless, mixture suppression was observed when equivalent dosages of either Z11-16:Ald (type A: 4/9 cases) or Z9-14:Ald (type B: 6/9 cases) were tested with a noncognate odorant (data not shown). It may also be the case that these studies vary due to our use of nonpheromonal odorants as components of mixtures.

Finally, the possibility for physical mixture interactions through pre-evaporative effects may also be occurring. Such pre-evaporative effects were described by Syed and Leal (2008) through chemical interactions with N,N-Diethyl-3-methylbenzamide (DEET). This seems unlikely in the current study, as other authors have demonstrated little evidence of pre-evaporative effect mixtures of naturally occurring compounds as tested in this study. Baker et al. (1998) found that mixtures of a pheromone blend attractive to *H. zea* (Z11-16:Ald + Z9-16:Ald) and Z11-16:OAc showed minimal differences in ratio of emission when compounds were emitted separately versus co-emitted from the same pipette. Furthermore, Fadamiro et al. (1999) tested this same combination of odorants and found the emission ratio of

Table 3 Mean latency in milliseconds from start of stimulation to stimulus-evoked spiking with varying ratios of odorant mixtures of insects with intact or transected antennal nerves (±standard error)

		X								
Type A		Z9-14:Ald	Z11-16:OAc	Z11-16:OH	16:OAc	16:OH	β-Caryophyllene	Linalool	Z3-06:OAc	Z3-06:OH
Intact	Z11-16:Ald	48 (4.3)	50 (4.7)	54 (5.5)	69 (7.2)	70 (9.5)	64 (6.6)	50 (5.3)	59 (6.6)	52 (6.3)
	1 μg X	54 (7.3)	51 (6.7)	64 (9.8)	64 (5.3)	78 (11.9)	57 (7.8)	69 (10.4)	66 (10.4)	65 (7.6)
	10 μg X	55 (7.3)	47 (4.9)	77 (12.3)	86 (14.9)	65 (7.9)	60 (7.8)	71 (8.4)	53 (5.6)	116 (18.0)
	100 μg X	56 (5.7)	43 (4.0)	68 (6.0)	103 (17.9)	73 (11.4)	68 (10.3)	60 (8.4)	74 (10.8)	96 (12.2)
Transected	Z11-16:Ald	102 (9.1)	88 (6.3)	100 (9.7)	100 (8.7)	89 (8.7)	99 (9.4)	108 (7.2)	99 (10.5)	112 (13.9)
	1 μg X	124 (12.8)	97 (7.7)	119 (12.2)	92 (9.8)	118 (10.8)	119 (13.3)	127 (9.9)	102 (12.5)	114 (16.9)
	10 μg X	126 (15.5)	126 (30.7)	146 (14.0)	117 (11.1)	128 (16.2)	121 (8.6)	124 (12.2)	124 (12.1)	158 (32.7)
	100 μg X	160 (15.8)	109 (11.4)	169 (18.8)	153 (30.4)	160 (15.7)	180 (29.9)	161 (15.6)	133 (11.3)	130 (18.6)
Type B		Z11-16:Ald	Z11-16:OAc	Z11-16:OH	16:OAc	16:OH	β-Caryophyllene	Linalool	Z3-06:OAc	Z3-06:OH
Intact	Z9-14:Ald	90 (5.1)	78 (5.4)	75 (3.5)	58 (8.1)	57 (10.9)	84 (4.9)	97 (6.9)	97 (6.6)	85 (6.7)
	1 μg X	132 (7.5)	106 (8.5)	105 (6.1)	118 (12.4)	83 (12.4)	97 (8.5)	124 (8.7)	122 (8.4)	124 (10.6)
	10 μg X	102 (5.1)	123 (10.5)	172 (16.6)	95 (11.2)	86 (9.6)	91.5 (6.2)	120 (8.6)	129 (10.6)	138 (9.2)
	100 μg X	135 (17.6)	146 (11.2)	176 (16.8)	128 (21.2)	75 (9.8)	135 (11.6)	201 (16.5)	125 (10.1)	146 (9.1)
Transected	Z9-14:Ald	94 (9.2)	74 (2.4)	77 (3.0)	67 (4.0)	79 (5.9)	72 (6.1)	74 (4.7)	97 (6.3)	98 (4.3)
	1 μg X	93 (8.8)	84 (3.4)	94 (2.8)	97 (7.8)	93 (10.6)	72 (7.2)	87 (5.5)	119 (8.9)	116 (6.2)
	10 μg X	120 (7.6)	83 (4.7)	133 (9.3)	81 (8.6)	98 (8.1)	86 (7.2)	100 (5.1)	107 (7.3)	129 (7.9)
	100 μg X	170 (11.9)	132 (17.0)	145 (16.5)	87 (7.9)	95 (13.2)	118 (10.1)	132 (10.4)	119 (9.2)	147 (9.9)

For each sensillar type, the first row represents latency following stimulation with the prime stimulus only (Z11-16:Ald or Z9-14:Ald) and subsequent rows represent increasing concentrations (1–100 μg) of a noncognate odorant in the mixture (X). Z3-06:OAc, Z3-hexenyl acetate; Z3-06:OH, Z3-hexen-1-ol.

pheromone (Z11-16:Ald + Z9-16:Ald) versus antagonist (Z11-16:OAc) issuing from separate pipettes was similar to emission from the same pipette.

No instances of linalool- or Z3-6:OH–produced synergy were observed when applied as a mixture with Z11-16:Ald, as observed in *H. zea* (Ochieng' et al. 2002). However, a small increase in spike frequency was noted following stimulation with β-caryophyllene + Z11-16:Ald. This difference in activity is interesting, as type A sensillar physiology appears homologous in these species, and indeed similarity between most of the heliothine moths studied to date, exhibiting a similar complement of Z11-16:Ald–sensitive long trichoid sensilla (Mustaparta 1997). If we assume that similar complements of binding proteins and receptors are present in each species' type A sensilla, how do variable responses to these mixtures arise? Type A sensilla in *H. virescens* (and *H. zea*; Lee et al. 2006a) have a second ORN present of unknown affinity; however, spike amplitudes in this study and cross-adaptation experiments by Ochieng' et al. (2002) indicate that the blend synergy observed in each case is isolated to the Z11-16:Ald–sensitive ORN. The mechanisms for differences in blend activation in these species have yet to be identified.

Spike frequency provides an absolute measure of odor input; however, timing of odorant activation is also important in signal interpretation and behavioral response. Latency to odor-evoked spiking was altered in most odor combinations tested in this study, in many cases shifting the physiological response by over 100 ms. This demonstrates that odor mixtures may alter the timing from stimulus delivery to impulse conduction from the sensillum to the AL and may vary according to the odor mixture and sensillar type. Such latency-intensity–dependent relationships have been demonstrated previously, in relation with intensity/concentration for a single stimulus (O'Connell 1975; Stange and Kaissling 1995). Given the fact that concentrations of cognate compounds were invariant in the current study, this effect may be attributed to noncompetitive inhibition, particularly as it was not observed during distal stimulation with noncognate odorants.

Electrophysiological and imaging studies have indicated that spike timing and glomerular activation time course differ according to odor activation, producing a spatial and temporal code (Lei et al. 2002). Shifting the timing of activation of a sensillar type in a population code may therefore produce "downstream" effects in synchronization and

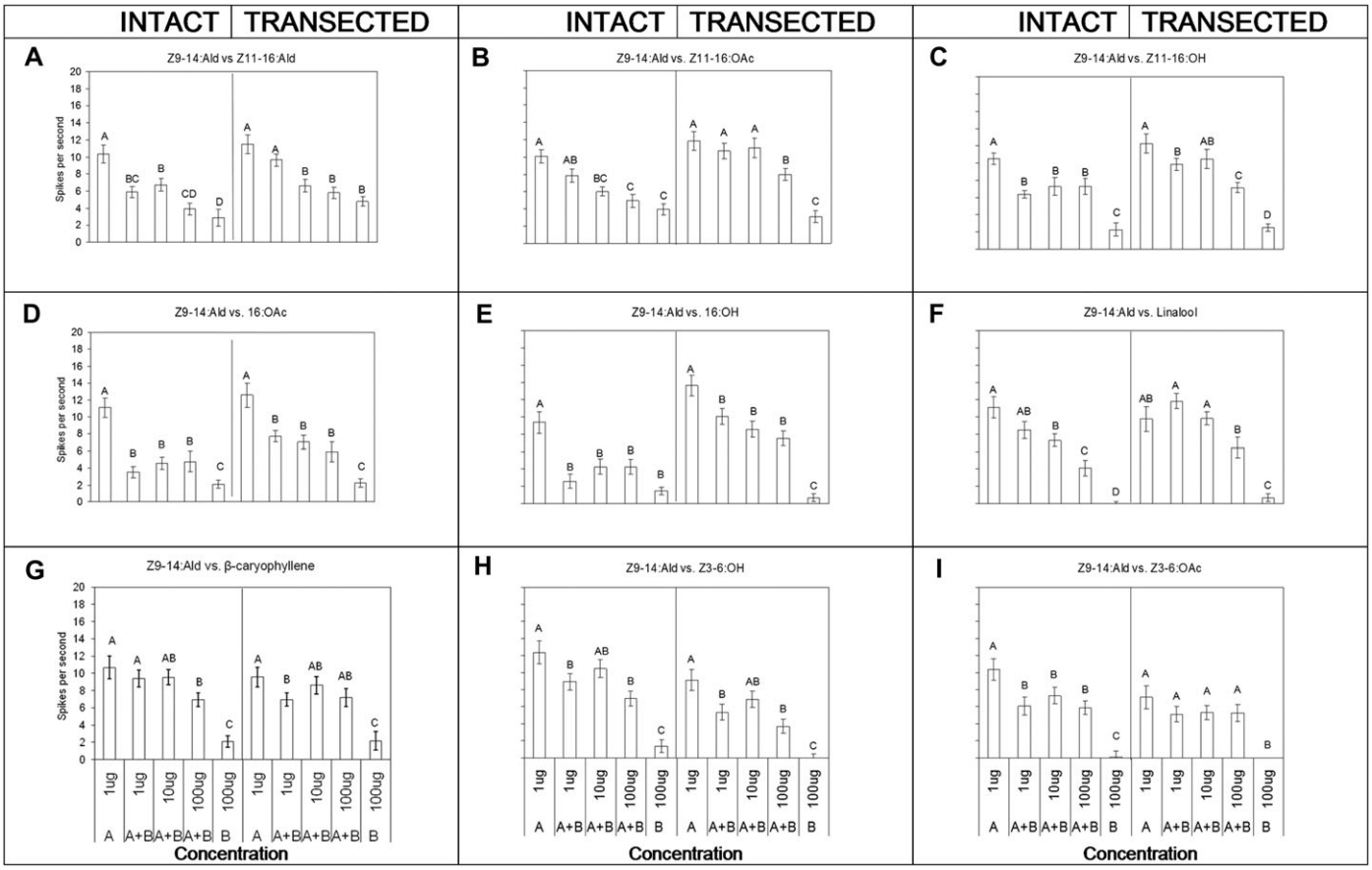

Figure 6 Type B sensillum—effects of odorant ratio in mixtures containing 1 μg Z9-14:Ald **(A)** and various noncognate odorants **(B)** on type B sensillum ORNs in preparations with intact or transected antennal nerves. In most cases, increases in the concentration of noncognate odorants mixed with Z9-14:Ald resulted in concentration-dependent decreases in ORN spiking relative to stimulation with Z9-14:Ald alone. Bars represented by different letters indicate significant differences within a treatment (intact or transected; Fishers LSD, $P < 0.05$).

temporal coding necessary for odor discrimination by the AL (Stopfer et al. 1997; Christensen et al. 1998). Additionally, moths following pheromone plumes must evaluate the composition and intermittency of blends rapidly to respond and engage in upwind anemotactic flight to locate an odor source (Vickers and Baker 1997). A slight shift in temporal integration of odor cues (as indicated by mixture suppression and latency effects) will likely affect an insect's ability to track a plume correctly.

Type A and B sensilla do not contain secondary ORNs that respond to the complement of odors tested; therefore, interactions between ORNs within a given sensillum can largely be dismissed (a second ORN is present in each with an unknown odorant affinity; Berg et al. 1998; Lee et al. 2006b). The soma of bipolar ORNs resides below the base of the sensillum near the antennal nerve (Keil 1999). As a consequence, many ORN cell bodies may be in close proximity to one another, along with a large population of axons from distal ORNs comprising the antennal nerve, providing an opportunity for ephaptic interactions between adjacent ORNs, or with the axon bundles of the antennal nerve (Vermeulen and Rospars 2004). This could explain inhibition produced by Z11-16:Ald or Z9-14:Ald, as the male antenna has a large number of ORNs that could produce considerable shifts in membrane potential (K^+_{out}) along the antennal nerve. Such effects have been investigated in the AL of *Manduca sexta*, wherein glial sheaths are believed to isolate ephaptic interactions to neurons within an individual glomerulus (Goriely et al. 2002). Stimulation of distal regions of the antenna with noncognate odorants showed no significant effect, however indicating that the observed mixture effects observed at the sensillum are not produced through interneuronal interactions along the antennal nerve.

Intact versus transected antennal nerve

Severing the antennal nerve affected a number of features associated with observed mixture responses in ORNs. In both sensillar types, prestimulus spontaneous spike activity was significantly reduced. Moreover, type A sensilla showed significantly increased latency to response in transected nerve preparations. It may be that there was a direct impact

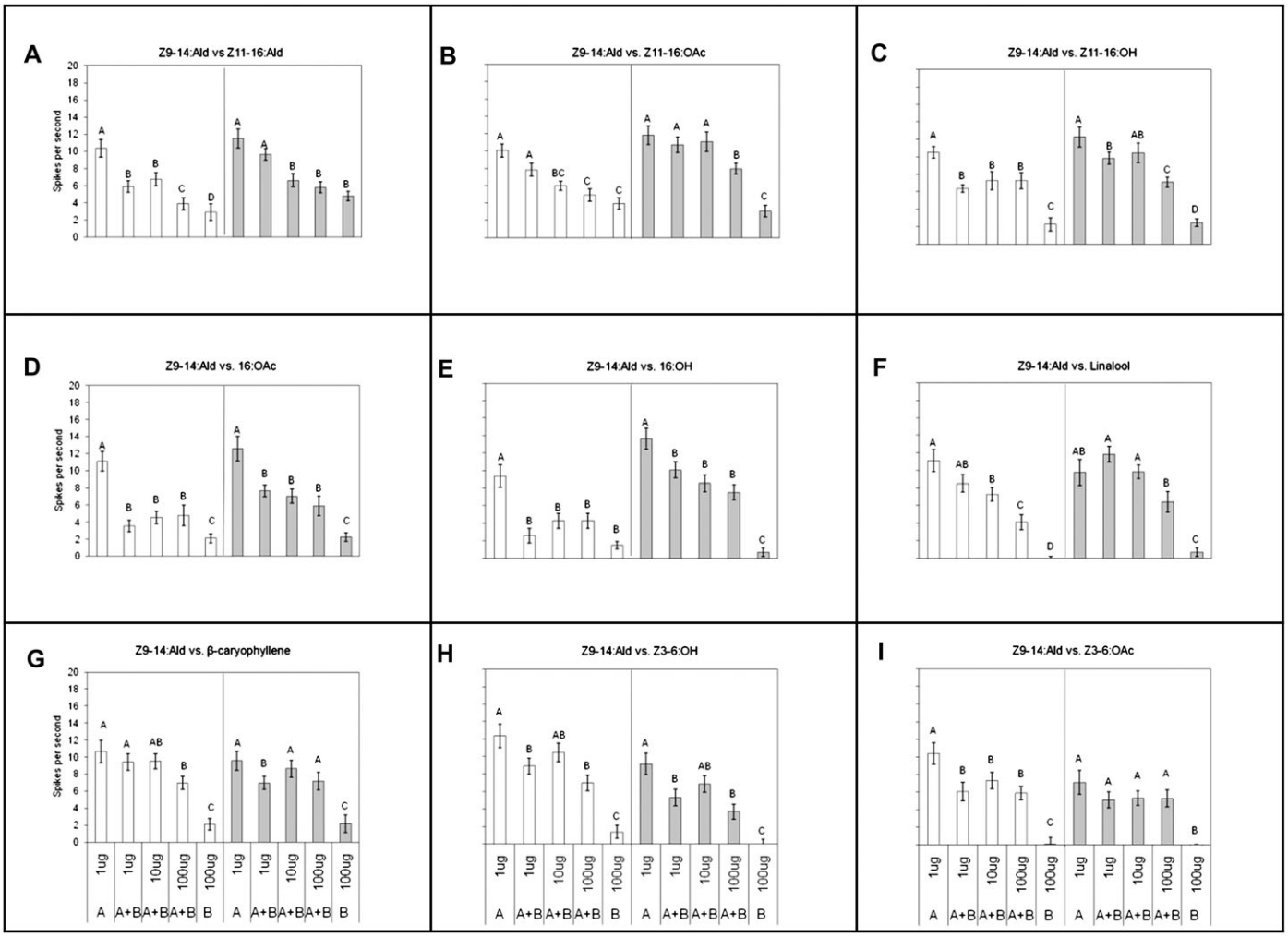

Figure 7 Type B sensillum—effects of antennal nerve transection on type B sensillum ORN responses to mixtures containing 1 μg Z9-14:Ald **(A)** and various noncognate odorants **(B)**. ORN activity was often increased in transected relative to intact preparations. White = intact antennal nerve, gray = transected antennal nerve. Asterisks indicate significant differences between intact and transected pairs of mixtures; Fishers LSD, $P < 0.05$.

upon ORN activity due to severing of the axon, and this direct damage impaired maintenance of ionic balance. The likelihood of cell damage or death significantly affecting recording seems unlikely during the time course of this experiment, as previous recordings have been made from severed antenna for considerably longer periods of time (Hillier NK, unpublished data). Alternatively, severing of the antennal nerve likely inhibited input to the antenna from sources in the AL.

Previous studies have demonstrated the importance of neuromodulators such as octopamine and serotonin in behavioral response and ORN activity (Linn and Roelofs 1986, 1992; Linn et al. 1992; Linn 1997; Pophof 2000; Dolzer et al. 2001). In the moth *Antheraea polyphemus* Cramer, octopamine was found to selectively increase spike frequency in an acetate-sensitive receptor, but not in an aldehyde receptor colocalized in the same sensillum (Pophof 2000). Grosmaitre et al. (2001) demonstrated similar increases in stimulus-evoked and spontaneous ORN spiking due to octopamine injection in *Mamestra brassicae*, along with inhibition in firing due to serotonin application. Additional research has demonstrated that both octopamine and serotonin exert effects on the transepithelial potential within sensilla (Dolzer et al. 2001). Octopamine receptors have been previously localized within *H. virescens* antenna (von Nickisch-Rosenegk et al. 1996), and transport of octopaminergic signals might be modulated by 2 separate mechanisms. First, it may be passed into the antenna via hemolymph circulation and octopamine secretion in the antennal heart (Pass et al. 1988). Second, it has been proposed that 1–2 octopamine-positive neurons may be present in *M. sexta* that project into the antennal nerve, though direct evidence of this seems limited (Dolzer et al. 2001). If octopamine is required to stimulate spontaneous firing in *H. virescens*, severing the antennal nerve might eliminate a neuronal source of input. In addition, our nerve transection protocol may have affected

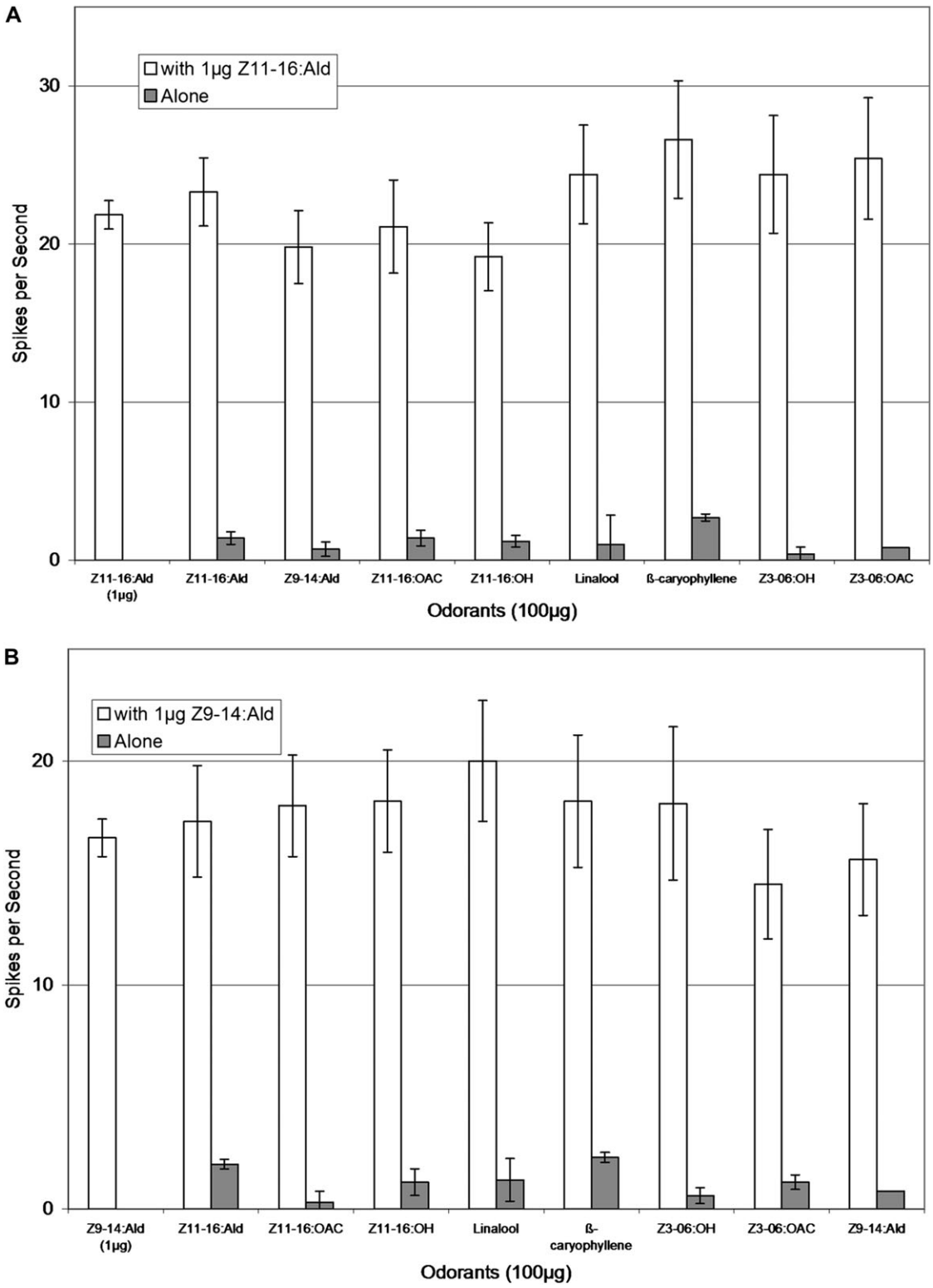

Figure 8 **(A)** Type A sensillar activity recorded during distal presentation of a series of odorants (100 µg) alone or during concurrent stimulation with 1 µg Z11-16:Ald ($F_{8,161} = 0.86$, $P = 0.54$); **(B)** type B sensillar activity recorded during distal presentation of a series of odorants (100 µg) alone or during concurrent stimulation with 1 µg Z9-14:Ald ($F_{8,161} = 0.45$, $P = 0.89$). No significant differences were noted through concurrent distal stimulation with various odorants versus proximal stimulation with Z11-16:Ald alone. Distal stimulation alone did not stimulate ORNs (with any of the odorants).

Table 4 Average latency to first spike (millisecond (±standard error)) following stimulus onset during odorant presentation to distal part of the antenna with or without concurrent stimulation at the proximal recording sensillum with 1 μg of Z11-16:Ald delivered to a type A sensilla or 1 μg of Z9-14:Ald delivered to a type B sensillum

	Type A ($F_{8,80}$ = 0.75, P = 0.64)	Type B ($F_{8,80}$ = 0.71, P = 0.68)
Stimulus	1 μg Z11-16:Ald	1 μg Z9-14:Ald
Z11-16:Ald (1 μg)	108 (27.0)	n/a
Z9-14:Ald (1 μg)	n/a	82 (14.0)
Distal odorants (100 μg)		
Z11-16:Ald	169 (29.0)	129 (58.0)
Z9-14:Ald	118 (63.0)	47 (1.0)
Z11-16:OAc	65 (11.0)	73 (19.0)
Z11-16:OH	96 (27.0)	100 (30.0)
Linalool	89 (33.0)	106 (26.0)
β-Caryophyllene	59 (9.0)	97 (26.0)
Z3-06:OH	59 (7.0)	81 (25.0)
Z3-06:OAc	53 (10.0)	47 (5.0)

No significant differences were found between sensillar responses to the cognate stimulus alone (i.e., Z11-16:Ald or Z9-14:Ald) or simultaneous presentation of odorants to the distal part of the antenna. Z3-06:OAc, Z3-hexenyl acetate; Z3-06:OH, Z3-hexen-1-ol; n/a, not applicable.

circulation by the antennal heart, thus preventing proper distribution of neuromodulators and oxygenation of ORNs along the antenna.

This study provides important considerations for future experimental design. First, stimulation with blends of odorants alters the activity of ORNs on the periphery relative to stimulation with solitary ligands, typically in an inhibitory fashion. The effects of blends depend upon the type of sensillum being tested, the additional components present in the mixture, and ratio of those components relative to the primary ligand. This should be considered in future electrophysiological investigation of sensillar or whole-antennal recording or in terms of behavioral testing of complex blends. Second, variation can exist between results obtained through protocols using intact or transected antennal preparations and may alter the response of individual ORNs to blends of odorants. This effect also varies according to sensillar type and odorant mixture. This is an important consideration, as many electrophysiological protocols (i.e., SSR, electroantennogram, and gas chromatography–electroantennographic detection) use antennae that have been completely removed from the insect.

The presence of odorant cues in mixtures of varying ratios may affect odor coding early in olfactory reception and processing. Interactions may therefore exist between odorants with very different behavioral relevance when present in a complex olfactory environment. Subsequently, this provides for an important consideration in evaluating behavioral and neurophysiological responses to odor blends.

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References

Ache BW. 1994. Towards a common strategy for transducing olfactory information. Semin Cell Biol. 5:55–63.

Akers RP, O'Connell RJ. 1988. The contribution of olfactory receptor neurons to the perception of pheromone component ratios in male redbanded leafroller moths. J Comp Physiol A. 163:641–650.

Atema J, Borroni PF, Johnson BR, Voigt R, Handrich L. 1989. Adaptation and mixture interactions in chemoreceptor cells: mechanisms for contrast enhancement. In: Laing DL, Cain W, McBride R, Ache BW, editors. Perception of complex smells and tastes. Sydney (Australia): Academic Press. p. 83–100.

Baker TC. 2009. Nearest neural neighbors: moth sex pheromone receptors HR11 and HR13. Chem Senses. 34:465–468.

Baker TC, Fadamiro HY, Cossé AA. 1998. Moth uses fine tuning for odour resolution. Nature. 393:530.

Berg BG, Almaas TJ, Bjaalie JG, Mustaparta H. 1998. The macroglomerular complex of the antennal lobe in the tobacco budworm *Heliothis virescens*: specified subdivision in four compartments according to information about biologically significant compounds. J Comp Physiol A. 183:669–682.

Bernays EA, Chapman RF. 1994. Host-plant selection by phytophagous insects. New York: Chapman and Hall.

Cardé RT. 1984. Chemo-orientation in flying insects. In: Bell WJ, Cardé RT, editors. Chemical ecology of insects. 1st ed. London: Chapman and Hall. p. 111–124.

Carlsson MA, Hansson BS. 2002. Responses in highly selective sensory neurons to blends of pheromone components in the moth *Agrotis segetum*. J Insect Physiol. 48:443–451.

Christensen TA, Waldrop BR, Hildebrand JG. 1998. Multitasking in the olfactory system: context-dependent responses to odors reveal dual GABA-regulated coding mechanisms in single olfactory projection neurons. J Neurosci. 18:5999–6008.

Cromarty SI, Derby CD. 1998. Inhibitory receptor binding events among the components of complex mixtures contribute to mixture suppression in responses of olfactory receptor neurons of spiny lobsters. J Comp Physiol A. 183:699–707.

Daniel PC, Burgess MF, Derby CD. 1996. Responses of olfactory receptor neurons in the spiny lobster to binary mixtures are predictable using a noncompetitive model that incorporates excitatory and inhibitory transduction pathways. J Comp Physiol A. 178:523–536.

De Moraes CM, Mescher MC, Tumlinson JH. 2001. Caterpillar-induced nocturnal plant volatiles repel conspecific females. Nature. 410:557–580.

den Otter CJ, Schuil HA, Sander-van Oosten A. 1978. Reception of host-plant odours and female sex pheromone in *Adoxophyes orana* (Lepidoptera: Tortricidae): electrophysiology and morphology. Entomol Exp Appl. 24:370–378.

Deng JY, Huang YP, Wei HY, Du JW. 2004. EAG and behavioral responses of *Helicoverpa armigera* males to volatiles from poplar leaves and their combinations with sex pheromone. J Zhejiang Univ Sci. 5:1577–1582.

Dolzer J, Krannich S, Fischer K, Stengl M. 2001. Oscillations of the transepithelial potential of moth olfactory sensilla are influenced by octopamine and serotonin. J Exp Biol. 204:2781–2794.

Fadamiro HY, Cossé AA, Baker TC. 1999. Fine scale resolution of closely space pheromone and antagonist filaments by flying *Helicoverpa zea*. J Comp Physiol A. 185:131–141.

Gödde J. 1989. Vibrating glass stylets: tools for precise microsurgery on cuticular structures. J Neurosci Methods. 29:77–83.

Goriely AR, Secomb TW, Tolbert LP. 2002. Effect of the glial envelope on extracellular K+ diffusion in olfactory glomeruli. J Neurophysiol. 87: 1712–1722.

Grosmaitre X, Marion-Poll F, Renou M. 2001. Biogenic amines modulate olfactory receptor neurons firing activity in *Mamestra brassicae*. Chem Senses. 26:653–661.

Hillier NK, Kleineidam CK, Vickers NJ. 2006. Physiology and glomerular projections of olfactory receptor neurons on the antenna of female *Heliothis virescens* (Lepidoptera: Noctuidae) responsive to behaviorally relevant odors. J Comp Physiol A. 192:199–219.

Hillier NK, Vickers NJ. 2004. The role of heliothine hairpencil compounds in female *Heliothis virescens* (Lepidoptera: Noctuidae) behavior and mate acceptance. Chem Senses. 29:499–511.

Hillier NK, Vickers NJ. 2007. Physiology and glomerular projections of sensilla on the antenna of *Heliothis virescens* responsive to behaviorally relevant odors II: male short trichoid sensilla. J Comp Physiol A. 193:649–663.

Howse P, Stevens I, Jones O. 1998. Insect pheromones and their use in pest management. London: Chapman and Hall. p. 369.

Kaissling K-E. 1974. Sensory transduction in insect olfactory receptors. In: Jaenicke L, editor. Biochemistry of sensory functions. Berlin (Germany): Springer. p. 243–273.

Kang JS, Caprio J. 1997. In vivo responses of single olfactory receptor neurons of channel catfish to binary mixtures of amino acids. J Neurophysiol. 77:1–8.

Keil TA. 1999. Morphology and development of the peripheral olfactory organs. In: Hansson B, editor. Insect olfaction. Berlin (Germany): Springer. p. 5–47.

Kurahashi T, Lowe G, Gold GH. 1994. Suppression of odorant responses by odorants in olfactory cells. Science. 265:118–120.

Lee S-G, Carlsson MA, Hansson BS, Todd JL, Baker TC. 2006a. Antennal lobe projection destinations of *Helicoverpa zea* male olfactory receptor neurons responsive to heliothine sex pheromone components. J Comp Physiol A. 192:351–363.

Lee S-G, Vickers NJ, Baker TC. 2006b. Glomerular targets of *Heliothis subflexa* male olfactory receptor neurons housed within long trichoid sensilla. Chem Senses. 31:821–834.

Lei H, Christensen TA, Hildebrand JG. 2002. Local inhibition modulates odor-evoked synchronization of glomerulus-specific output neurons. Nat Neurosci. 5:557–565.

Linn CE. 1997. Neuroendocrine factors in the photoperiodic control of male moth responsiveness to sex pheromone. In: Cardé RT, Minks AK, editors. Insect pheromone research. New directions. New York: Chapman and Hall. p. 194–209.

Linn CE, Campbell MG, Roelofs WL. 1992. Photoperiod cues and the modulatory action of octopamine and 5-hydroxytryptamine on locomotor and pheromone response in male gypsy moths *Lymantria dispar*. Arch Insect Biochem Physiol. 20:265–284.

Linn CE, Roelofs WL. 1986. Modulatory effects of octopamine and serotonin on male sensitivity and periodicity of response to sex pheromone in the cabbage looper moth *Trichoplusia ni*. Arch Insect Biochem Physiol. 3: 161–172.

Linn CE, Roelofs WL. 1992. Role of photoperiodic cues in regulating the modulatory action of octopamine on pheromone-response threshold in the cabbage looper moth. Arch Insect Biochem Physiol. 20:285–302.

Lucero MT, Horrigan FT, Gilly WF. 1992. Electrical responses to chemical stimulation of squid olfactory cells. J Exp Biol. 162:231–249.

Mayer MS, McLaughlin JR. 1995. Handbook of insect pheromones and sex attractants. Boca Raton (FL): CRC Press. p. 1083.

Michel WC, McClintock TS, Ache BW. 1991. Inhibition of lobster olfactory receptor cells by an odor-activated potassium conductance. J Neurophysiol. 65:446–453.

Mustaparta H. 1997. Olfactory coding mechanisms for pheromone and interspecific information in related species of moths. In: Cardé RT, Minks AK, editors. Insect pheromone research. New directions. New York: Chapman and Hall. p. 144–163.

Nikonov A, Leal W. 2002. Peripheral Coding of Sex Pheromone and a Behavioral Antagonist in the Japanese Beetle, *Popillia japonica*. J Chem Ecol. 28:1075–1089.

Ochieng' SA, Park KC, Baker TC. 2002. Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male *Helicoverpa zea*. J Comp Physiol. 188:325–333.

O'Connell RJ. 1975. Olfactory receptor responses to sex pheromone components in the red-banded leaf roller moth. J Gen Physiol. 65. 179–205.

O'Connell RJ, Akers RP. 1989. Responses of insect olfactory receptor neurons to biologically relevant mixtures. In: Laing DG, Cain WS, McBride RL, Ache BW, editors. Perception of complex smells and tastes. San Diego (CA): Academic Press. p. 49–63.

O'Connell RJ, Beauchamp JT, Grant AJ. 1986. Insect olfactory receptor responses to components of pheromone blends. J Chem Ecol. 12:451–467.

Oka Y, Omura M, Kataoka H, Touhara K. 2004. Olfactory receptor antagonism between odorants. EMBO J. 23:120–126.

Olson KS, Derby CD. 1995. Inhibition of taurine and 5'AMP olfactory receptor sites of the spiny lobster *Panulirus argus* by odorant compounds and mixtures. J Comp Physiol A. 176:527–540.

Pass S, Sperk G, Agricola H, Baumann E, Penzlin H. 1988. Octopamine in a neurohaemal area within the antennal heart of the American cockroach. J Exp Biol. 135:495–498.

Pophof B. 2000. Octopamine modulates the sensitivity of silkmoth pheromone receptor neurons. J Comp Physiol A. 186:307–313.

Roelofs WL, Hill AS, Cardé RT, Baker TC. 1974. Two sex pheromone components of the tobacco budworm moth, *Heliothis virescens*. Life Sci. 14:1555–1562.

Sanhueza M, Schmachtenberg O, Bacigalupo J. 2000. Excitation, inhibition, and suppression by odors in isolated toad and rat olfactory receptor neurons. Am J Physiol Cell Physiol. 279:C31–C39.

Shields VDC, Hildebrand JG. 2001. Responses of a population of antennal olfactory receptor cells in the female moth *Manduca sexta* to plant-associated volatile organic compounds. J Comp Physiol A. 186: 1135–1151.

Shorey HH, Hale RL. 1956. Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. J Econ Entomol. 58:522–524.

Stange G, Kaissling KE. 1995. The site of action of general anaesthetics in insect olfactory receptor neurons. Chem Senses. 20:421–432.

Steullet P, Derby CD. 1997. Coding of blend ratios of binary mixtures by olfactory neurons in the Florida spiny lobster, *Panulirus argus*. J Comp Physiol A. 180:123–135.

Stopfer M, Bahgavan S, Smith BH, Laurent G. 1997. Impaired odour discrimination on desynchronization of odour-encoding neural assemblies. Nature. 390:70–74.

Syed Z, Leal WS. 2008. Mosquitoes smell and avoid the insect repellent DEET. Proc Natl Acad Sci USA. 105:13598–13603.

Teal PEA, Tumlinson JH. 1989. Isolation, identification and biosynthesis of compounds produced by male hairpencil glands of *Heliothis virescens* (F.) (Lepidoptera: Lepidoptera). J Chem Ecol. 15:413–427.

Van der Pers J, den Otter CJ. 1978. Single cell responses from olfactory receptors of small ermine moths to sex-attractants. J Insect Physiol. 24:337–343.

Vermeulen A, Rospars J-P. 2004. Why are insect olfactory receptor neurons grouped into sensilla? The teachings of a model investigating the effects of the electrical interaction between neurons on the transepithelial potential and the neuronal transmembrane potential. Eur Biophys J. 33: 633–643.

Vetter RS, Baker TC. 1983. Behavioral responses of male *Heliothis virescens* in a sustained-flight tunnel to combinations of seven compounds identified from female sex pheromone glands. J Chem Ecol. 9:747–759.

Vickers NJ, Baker TC. 1997. Flight of *Heliothis virescens* males in the field in response to sex pheromone. Physiol Entomol. 22:277–285.

von Nickisch-Rosenegk E, Krieger J, Kubick S, Laage R, Strobel J, Strotmann J, Breer H. 1996. Cloning of biogenic amine receptors from moths (*Bombyx mori* and *Heliothis virescens*). Insect Biochem Mol Biol. 26:817–827.

Witzgall P, Lindblom T, Bengtsson M, Tóth M. 2004. The pherolist [Internet]. IOBC Working Group "*Pheromones and Other Semiochemicals in Integrated Production*". [Cited 2010 May 20]. Available from: URL http://www-pherolist.slu.se/pherolist.php.