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Pheromone behavioral responses in unusual male European corn borer hybrid progeny not correlated to electrophysiological phenotypes of their pheromone-specific antennal neurons

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Abstract. In genetic studies on the sex pheromone communication system of two races of European corn borer, which use opposite pheromone blends of the E and Z compounds, it was found that antennal olfactory cell response amplitudes to the two compounds were controlled by an autosomal factor, whereas behavioral responses to the blends were controlled by a sex-linked locus. Because of the difference in genetic controls, it was postulated that some unusual males would be produced in F_2 crosses between these two races. These unusual males would have antennal olfactory cells that respond as the Z-race males, but would respond behaviorally to the E blend. The present studies combined behavioral studies in a flight tunnel and single cell electrophysiological studies to show that these unusual males do indeed exist. These findings show that the spike amplitude of peripheral olfactory cells is not important in regulating species- or race-specific pheromone responses, as compared to some central nervous system factor assesses the spike frequencies from different pheromone-component-specific cells on the antenna. This factor seems to be essential in governing the pheromone-blend specific behavioral responses of male moths.
Key words. European corn borer; *Ostrinia nubilalis*; flight tunnel; single-cell electrophysiology; genetics.

The European corn borer (ECB), *Ostrinia nubilalis*, in New York exhibits polymorphisms that include differences in the sex pheromone communication system. In the Z races (bivoltine and univoltine), females emit and males respond to a 3:97 sex pheromone blend of (E)-/(Z)-11-tetradecenyl acetates (E11-/Z11-14:OAc)[1], and in the E race (bivoltine only), females emit and males respond to the opposite 99:1 E/Z pheromone blend[2]. Hybrid individuals can be produced in the laboratory and are found in areas where the two races occur in sympatry[3]. Analysis of hybrid females from reciprocal crosses has shown that they produce an intermediate E11-/Z11–14:OAc pheromone blend of 65:35, but males respond equally to blends containing 3–65% E isomer[4,5]. The two pheromone components are detected by different specialized receptors in the olfactory sensilla on the male antennae[6]. In each sensillum Z race males have a receptor cell characterized by a large spike amplitude tuned to the Z-isomer. In E race males the situation is the reverse; a large spike amplitude cell is tuned to the E isomer and a small spike amplitude cell to the Z isomer. In hybrids of the E and Z race the spike amplitudes are of equivalent height. A third cell responds to (Z)-9-tetradecenyl acetate (Z9–14:OAc) in both the parental races and in the hybrids[6]. This compound has been found to be a behavioral antagonist with ECB[7,8].

Genetic studies have shown that female production of the final blend ratio is under control of a major autosomal locus[4,9], but that the sequence of male upwind flight responses to pheromone is controlled by a sex-linked locus[4] (males are the homogametic sex). Sex-linked control of behavioral responses in crosses of E and Z ECB was confirmed by demonstrating complete linkage of a sex-linked TPI (triose phosphate isomerase) locus and the locus controlling response to sex pheromone[10]. However, it was found from electrophysiological recordings with single olfactory sensilla on male antennae[4], that the patterns of spike action potential amplitudes in response to pheromone components are controlled by an autosomal factor lacking dominance. In summary, the loci for pheromone production, antennal olfactory cell response amplitude, and behavioral response are independently inherited (the first two on autosomes, the third on the Z chromosome), with no apparent linkage between the autosomal factors since it was found[11] that they independently assort.

Normally, males of one pheromone race do not respond to the blend of the other race. However, in crosses between E and Z races, inheritance patterns in F_2 male ECB of the upwind-flight responses (sex-linked control) and of the male antennal olfactory cell responses (autosomal control) reveal that some unusual males should be produced that respond behaviorally to one blend, but possess antennae that respond electrophysiologically to the opposite blend. The behavioral-response profile of F_2 males (fig. 1) generated from an initial E(male) × Z(female) cross shows that it is composed of

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a 1:1 mixture of hybrid-responders and E-responders[4,10]. The hybrid males respond in the flight tunnel to a range of blends from 65:35 E/Z to 3:97, but not to the E blend[4,5], whereas the E males respond behaviorally mainly to the E blend, and not to the Z blend. The electrophysiological responses (spike action potential amplitudes) at the sensillum level of these F_2 males, however, are under autosomal control and, thus, are inherited in a 1:2:1 pattern (E:hybrid:Z) independent of the F_2 behavioral phenotypes (fig. 1). F_2 males that fly to the E blend, but possess antennae that are either hybrid ($Z^EZ^EA^EA^Z$) or Z ($Z^EZ^EA^ZA^Z$), would be unusual since males possessing these antennae normally do not respond behaviorally to the E blend. F_2 males that respond behaviorally to the Z blend (hybrid males), but possess E antenna ($Z^EZ^ZA^EA^E$) would be unusual since males with E antennae normally do not respond to the Z blend.

The present study was undertaken to determine if an unusual male can complete a full sequence of behavioral responses to a blend when they possess the wrong antennae. In addition, we wished to determine any effects on spike frequency relative to spike amplitude for the major and minor pheromone components in the specialized olfactory cells of the unusual males.

Materials and methods

The Z race of ECB was maintained at the Corn Insect Research Laboratory, USDA, Ames, Iowa. The colony was established with adults collected from cornfields in several areas in and around Ames, Iowa, during the spring of 1992. The E race colony was established from larvae collected from corn stubble in several areas of New York where it was known to be predominant and maintained using techniques and diet identical to our previous studies[2,3]. Crosses were carried out by placing 80 E males and 60 Z females in cylindrical screen cages and collecting eggs from waxed paper lining the cages. After hatching, larvae were reared at 30 °C with a 16:8 L:D photoperiod. Over 2000 F_1 pupae were obtained, and about 1/4 of them used to generate the F_2 progeny in a similar manner. Male F_2 pupae were isolated individually in 35.5 cc plastic cups and held at 25 °C on a 14:10 L:D photoperiod until the adults were 1–2 days old. Over 1500 male F_2 pupae were obtained, and about 1/3 of them were set up for behavioral analysis in the flight tunnel.

Behavioral responses. Flight tunnel bioassays were conducted using a previously described flight tunnel[2]. The wind speed during flights was 0.4 m/s and the illumination was 5 lux of red light on the tunnel floor. The distance from the pheromone source to the insect release site was 1.2 m. Temperature was maintained at 19–21 °C. Relative humidity was not controlled and varied from 36%–79%. The pheromone blends were prepared as described previously[7]. The standard Z blend consisted of 3:97 E11-/Z11-14:OAc and the standard E blend consisted of 99:1 E11-/Z11-14:OAc. Lures were made by applying 30 µl of hexane containing 30 µg of a blend to a 5 × 9 mm rubber stopper (Thomas Scientific, Swedesboro, NJ, Cat. No. 8753-D22). Each stopper was allowed to stand in a hood 2–4 h before initial use and was stored in a screwtop glass vial at −20 °C. The lures were warmed to room temperature prior to introduction into the flight tunnel.

Male European corn borer pupae and newly eclosed adults were held at 25 ± 1 °C on a 14:10 L:D photoperiod until the adults were 1–2 days old. For testing, each insect was placed in a glass jar in the flight tunnel room, 30 min before scotophase. Between 3–5 h into scotophase, individual males were placed in the flight tunnel on a 10 cm high release platform in a 3 cm × 6 cm screen cylinder with the open end upwind. Each insect was allowed 30–60 s to initiate flight. Insects that did not respond in the time allotted, but could fly, were recorded as nonresponders (NR). Behavioral responses were recorded as follows: ACT, activation, rapid wing beating and walking; TF, taking flight; CA, casting flight; OR, orientation flight in the odor plume; UP, upwind flight; 10 cm, flight to 10 cm from the pheromone source; TS, touching or landing on the source; DS, display, clasper extrusion with wings held vertically and abdomen waving slowly from side to side.

All males on a given day were given a chance to respond by flying in response to one of the standard pheromone blends, recaptured and approximately 30 min after the initial flight, were tested again to the opposite blend. Every other day the order in which the blends were used was reversed. Z-strain males were also flown daily to the Z blend pheromone as a control. A total of 186 insects were tested to the two blends. Tests were conducted between July 14, 1993 and August 19, 1993 and between November 10, 1993 and December 2, 1993.

Only males flying upwind to within 10 cm of the pheromone source, including either touching the source or displaying, were considered to have a positive response to that blend. Behavioral response phenotypes were assigned as follows: individual insects responding to neither blend were recorded as nonresponders, those responding to the E blend and not the Z blend were recorded as E behavioral types, those responding to the Z blend and not the E blend were recorded as hybrid behavioral types, and those responding to both were recorded as E & H behavioral types. It should be noted that only E and hybrid males should be produced in this particular F_2 progeny (see fig. 1) and hybrid males respond to the Z blend but not to the E blend. Successful behavioral analyses were completed on 171 males. These males were placed individually in 35.5 cc plastic cages with dental wicks moistened with distilled H_2O. The cups were packed with artificial ice in polystyrene

	Females	x	Males	
Parents	Z^ZW	A^ZA^Z	Z^EZ^E	A^EA^E
F_1	Z^EW	A^EA^Z	Z^EZ^Z	A^EA^Z
F_2			1/8 Z^EZ^E	A^EA^E
			2/8 Z^EZ^E	A^EA^Z
			1/8 Z^EZ^E	A^ZA^Z
			1/8 Z^EZ^Z	A^EA^E
			2/8 Z^EZ^Z	A^EA^Z
			1/8 Z^EZ^Z	A^ZA^Z

Figure 1. The expected genotypes for sex-linked genes controlling male behavioral response and for autosomal genes controlling male sensillum type from an initial cross of Z race females and E race males. The large Z's and W's represent sex chromosomes and the large A's represent autosomes. The small Z's and E's represent alleles originating in the Z or E race, respectively. In the F_2 progeny, only the male genotypes are presented.

peanuts in coolers and shipped overnight to Iowa State University where electrophysiological recordings were obtained on 144 of them.

Electrophysiological recordings. To record from the olfactory receptor cells within individual antennal sensilla, a cut-sensillum technique developed by Kaissling[12,13] was used. Briefly, the right antenna of a moth was excised from the head, and the antennal base was placed in a saline-filled Ag/AgCl pipette recording electrode. Using a micromanipulator, the antenna was maneuvered 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 placed in a second micromanipulator, and the cut end was contacted with a second saline-filled Ag/AgCl pipette recording electrode. Two sensilla were chosen at random from accessible areas of the antenna, one for the actual recording and the other one to verify the recorded responses. No instances of conflicting response profiles were noted between any such pair of sensilla on a single antenna. This technique has proved to be an accurate method of sensilla sampling based on inheritance ratios in past crosses[6]. The antenna was continuously bathed in a stream of purified, humidified air (10 ml/s) that passed through a glass tube (8 mm i.d.) whose outlet was positioned 2 cm from the antenna. Prior to and after exposure to any of the test compounds, the spontaneous activities of the receptor cells within each sensillum were monitored over a 5 s period. With but a few exceptions, the recordings showed a complete lack of spontaneous activity.

Each of the three acetates was purchased from the pheromone library at the Institute for Pesticide Research, Wageningen, The Netherlands. Serial dilutions of each chemical were prepared in HPLC grade hexane. The E11–14:OAc contained 1% of the Z-isomer, and purities of Z11–14:OAc and Z9–14:OAc were 100% as verified by capillary GLC (DB-5, DB-225, J & W Scientific, Folsom, California). Solutions were stored in 3.7 ml glass vials at −20 °C. For each of the compounds tested, 10 µg of a diluted solution was pipetted onto a filter paper strip held in a Pasteur pipette glass cartridge. Preliminary experiments using a 1 µg dosage showed spike amplitude and spike frequency profiles to be similar to those elicited by 10 µg, but far fewer spikes were recorded from only a small percentage of antennae. At a dosage of 100 ng, virtually no responses could be recorded from any antennae. To increase the sample pool, the dosage was set at 10 µg. Receptor cells were exposed to 20 ms puffs[14] of each of the three acetates in random order by manually injecting a 2 ml puff into the airstream through a hole in the glass tube 15 cm from the outlet. Between 10 and 30 s elapsed between puffs. Since the absolute spike amplitude and spike frequency are dependent on the quality of the electrode connection and vary from male to male, relative numbers were used to present the data most reliably.

The electrical responses of the receptor neurons were recorded using a differential AC Grass P15 preamplifier (Grass Instruments, Quincy, Massachusetts), and monitored visually on a Gould 1604 digital storage oscilloscope (Gould Inc., Cleveland, Ohio). They were then recorded onto video tapes 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 Metra-Byte Corp., Taunton, Massachusetts) running SAPID software[15]. The amplified ($\times 1000$) and filtered (100–10,000 Hz) analog 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 sort spikes according to mean action potential amplitudes and to count the number of spikes in each class, using the entire spike train from each cell.

Results

F_2 males were selected with a stringent behavioral-response protocol in the flight tunnel in which they were required to fly all the way to the E or Z source to be classified as an E or hybrid male, respectively. Of the 171 males tested, 73 were classified as E-responders, 38 as hybrid, 54 as nonresponders, and 6 responded to both E and Z blends. In previous studies[4] it was found that only ca. 50% of hybrid males reach the source compared to 90% for E males with the protocol used. Thus, the numbers are close to the expected 1:1 ratio

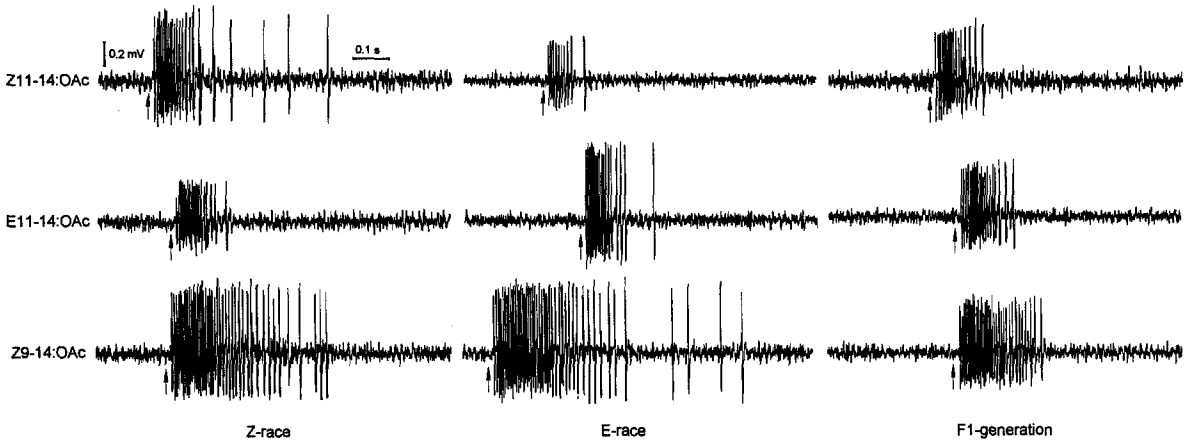

Figure 2. Typical single sensillum responses from olfactory sensilla on male *O. nubilalis* antennae in the Z and E races and in the (Z × E)F_1 hybrids to Z11–14:OAc, E11–14:OAc, and Z9–14:OAc. Arrows represent stimulus presentation.

(fig. 1) of E/hybrid if adjustments are made for the nonresponders in each class.

Electrophysiological recordings from single olfactory sensilla on male antennae showed that the response profiles of the parental Z and E races, and F_1 hybrids with regard to both spike height and spike frequency were easily distinguishable (fig. 2). In both the parental races, each hair contained a large spike amplitude cell that responded with a high spike frequency to the main sex pheromone component of the race, and a small spike amplitude cell that responded with a lower spike frequency to the minor pheromone component. Antennae that contained such hairs were called Z- and E-type antennae. In the F_1 hybrids, the two pheromone components elicited equal amplitude spikes and equal spike frequencies and 'intermediate' antennae on which such hairs existed were call I-type antennae. A third, large amplitude cell housed in these same hairs responded with a high spike frequency to Z9–14:OAc in both parental races and in the hybrid.

With the Z-race males the difference in spike amplitude between the two cells responding to the two pheromone components was ca. 18%, whereas the cells in the E race showed a difference of ca. 34%. The spike amplitudes elicited by Z11–14:OAc and E11–14:OAc in the F_1 hybrid males differed by less than 3% (fig. 3A). With the E-race males the difference in spike amplitude between cells firing in response to the main pheromone component and those responding to Z9–14:OAc was ca. 16%, whereas with the Z race males and F_1 hybrid males the amplitude differences were less than 3% between cells responding to Z11–14:OAc vs. Z9–14:OAc (fig. 3B).

The relative differences of spike frequency responses of the parental Z and E race neurons and those of the F_1 hybrids when presented with the two pheromone components were very similar to the proportional differences in spike amplitude (fig. 4A). The main pheromone component of each of the parental races elicited relatively high spike frequencies in neurons from males of

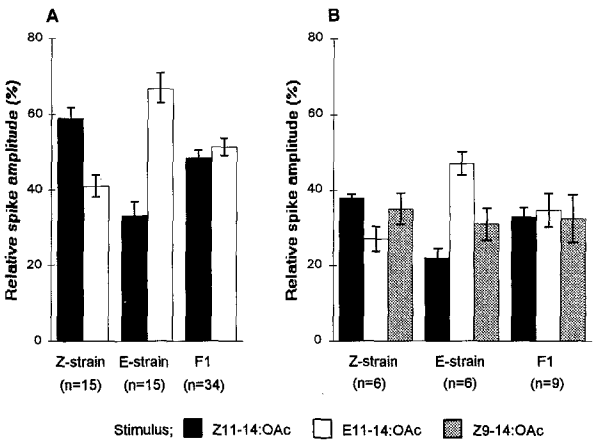

Figure 3. Relative spike amplitude differences among spikes elicited from three receptor neurons present in male *O. nubilalis* antennal sensilla by Z11–14:OAc, E11–14:OAc, and Z9–14:OAc for the Z race, E race, and (Z × E)F_1 generation. Relative spike amplitude was calculated by taking the mean amplitude elicited by: *A* the two different receptor neurons tuned to either Z11–14:OAc or E11–14:OAc; or *B* the three different receptor neurons tuned to either Z11–14:OAc, E11–14:OAc, or Z9–14:OAc, and dividing them by the sum of the mean amplitudes elicited by those three compounds. Standard deviations of percentages are presented as error bars.

the corresponding strain compared to frequencies evoked by the minor component, whereas equivalent spike frequencies were elicited by the major and minor components in neurons from F_1 hybrids. Interestingly, the highest spike frequencies were recorded in response to Z9–14:OAc in all three genotypes (fig. 4B). The response profiles in figures 3A and 4A were used for analysis of the F_2 generation males that had been tested in the flight tunnel and assigned to a behavioral class.

Electrophysiological recordings from single olfactory sensilla on male antennae were conducted successfully on 144 males, including 62 E-behavioral responders, 32 hybrid-behavioral responders, 44 nonresponders, and 6 that responded to both E- and Z-sources. Spike amplitude analyses (fig. 5) of the E-behavioral responders

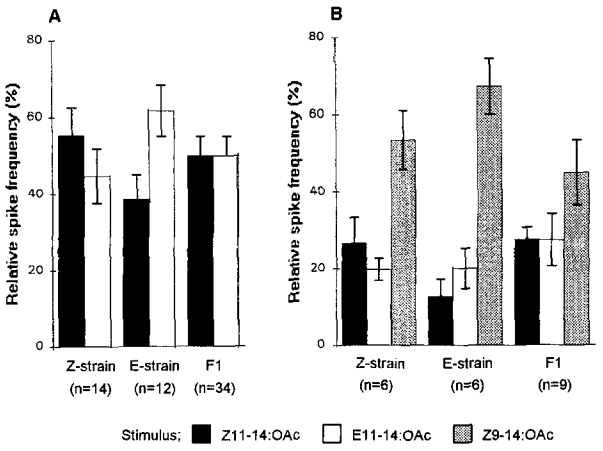

Figure 4. Relative spike frequency difference among spikes elicited from three receptor neurons present in male *O. nubilalis* antennal sensilla by Z11–14:OAc, E11–14:OAc, and Z9–14:OAc for the Z race, E race, and $(Z \times E)F_1$ generation. Relative spike frequency was calculated by taking the mean number of spikes/s elicited by *A* the two different receptor neurons tuned to either Z11–14:OAc or E11–14:OAc, or *B* the three different receptor neurons tuned to either Z11–14:OAc or E11–14:OAc or Z9–14:OAc, and dividing them by the sum of the mean number of spikes/s of Z11–14:OAc, E11–14:OAc, and Z9–14:OAc. Standard deviations of percentages are presented as error bars.

revealed a composition of 39 males with E-type, 15 with Intermediate-type (I-type), and 8 with Z-type antennae. A similar distribution was found for the hybrid-behavioral responders (17 E-types, 10 I-types, and 5 Z-types) and for the nonresponders (21 E-types, 16 I-types, and 7 Z-types). With the 6 males (not shown) that completed flights to both the E race pheromone source and the Z-race pheromone source, only E-type (5) and I-type (1) antennae were found.

Similar antennal-type ratios were found for all 4 behavioral response types when spike frequency was used as an analytical criterion (fig. 6). In this case, spike amplitude was ignored completely and only firing frequencies in response to either Z11–14:OAc or E11–14:OAc were calculated. Based on this criterion, the ratios of firing showed again that the majority of F_2 males possessed E-type antennae with the exception of the nonresponders in which there were 19 E-type antennae and 20 males with I-type antennae. Also all 6 of the males that responded to both Z and E blends carried E-type antennae (not shown). The majority of males with E-type antennae based on spike amplitude were also the same males judged as having E-type antennae based on spike

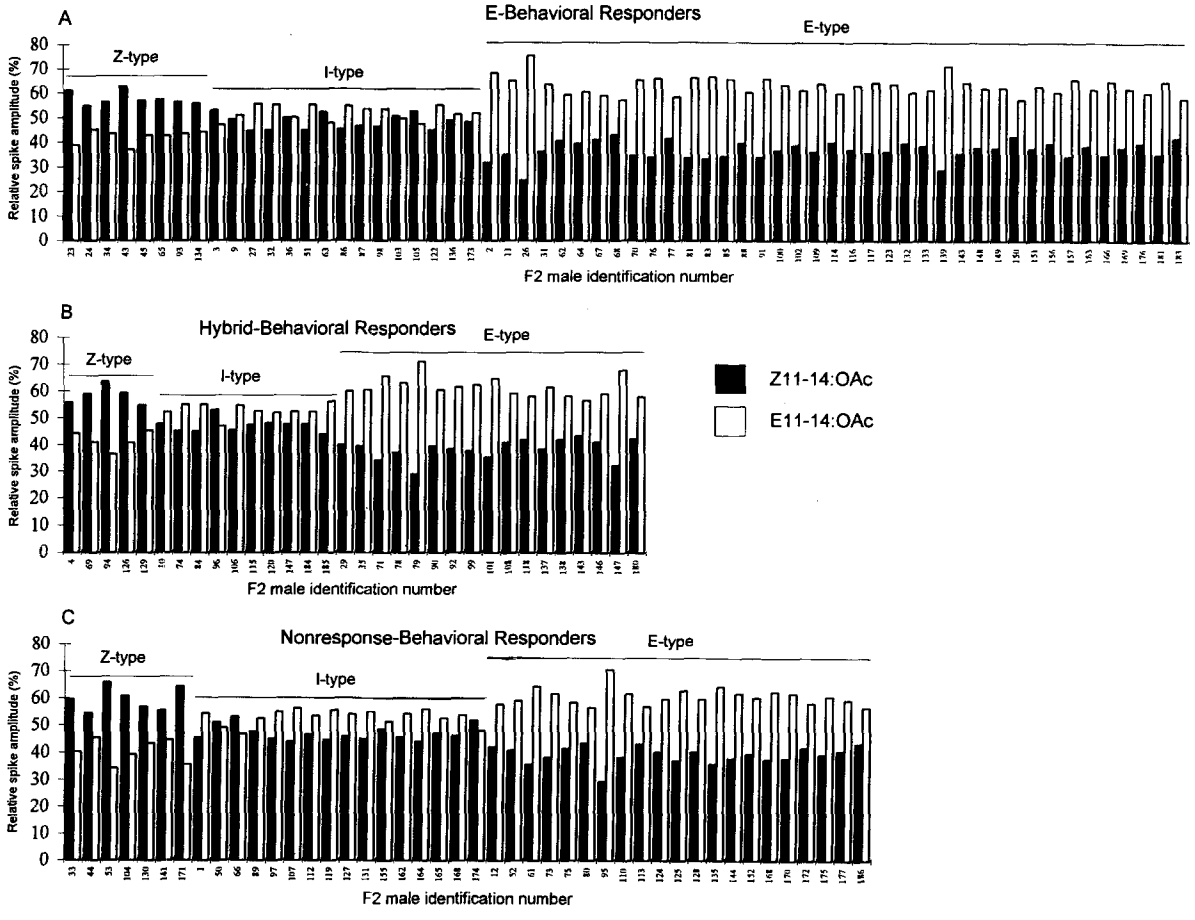

Figure 5. Electrophysiological phenotypes of $(Z \times E)F_2$ *O. nubilalis* males based on the relative spike amplitude difference between spikes elicited from two receptor neurons by Z11–14:OAc and E11–14:OAc for *A* E-behavioral responders, *B* hybrid-behavioral responders, and *C* nonresponders. Phenotype classifications were calculated using a t-test for differences between the amplitudes of a single observation and the mean amplitudes of the F_1 and the parental Z and E races at $p \geq 0.05$[27].

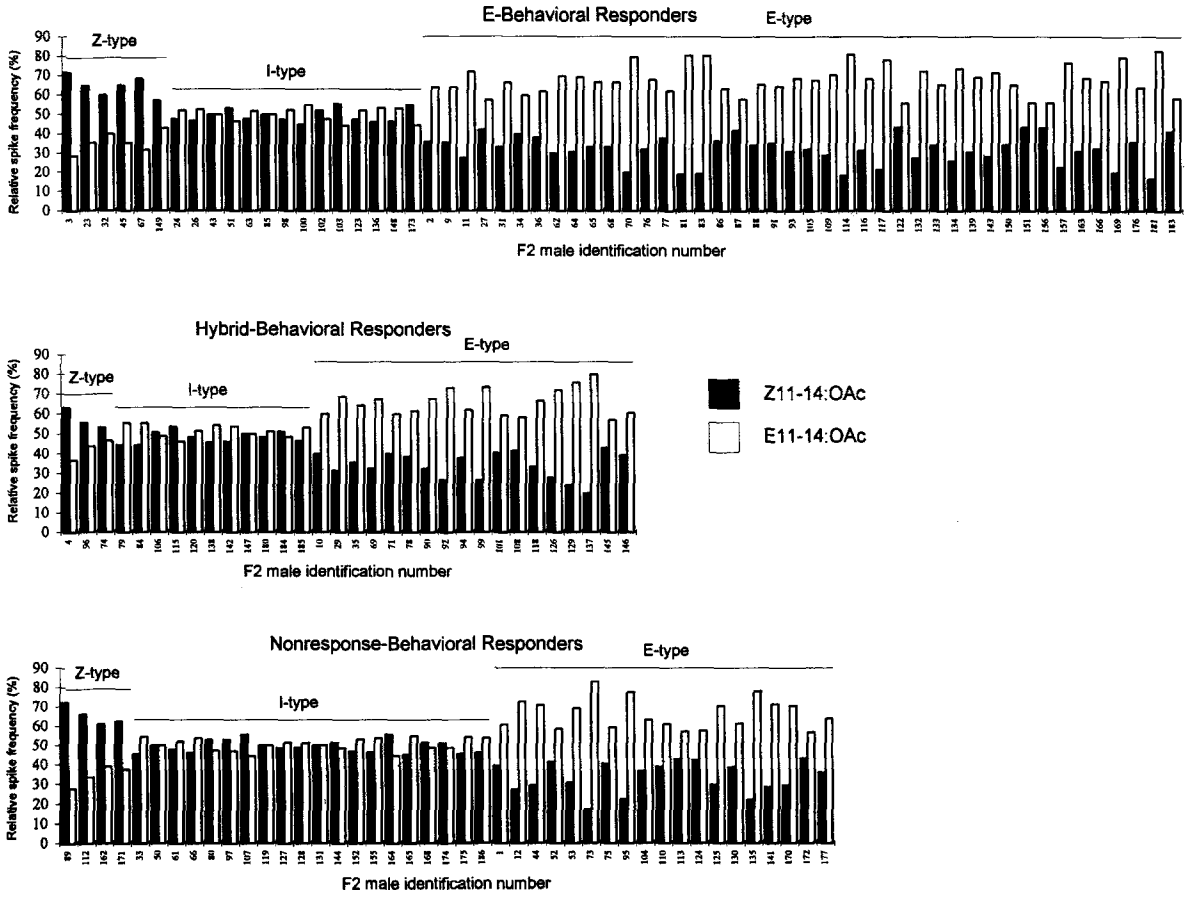

Figure 6. Electrophysiological phenotypes of $(Z \times E)F_2$ *O. nubilalis* males based on the relative spike frequency difference between spikes elicited from two receptor neurons by Z11–14:OAc and E11–14:OAc for *A* E-behavioral responders, *B* hybrid-behavioral responders, and *C* nonresponders. Phenotype classifications were calculated using a t-test for differences between the amplitudes of a single observation and the mean amplitudes of the F_1 and the parental Z and E races at $p \geq 0.05$[27].

frequency (E-beh. 31/39, hybrid-beh. 12/17, nonresponders-beh 13/21, and E & H-beh. 5/6). Similar correlations between spike amplitude and spike frequency were found for those F_2 males carrying I-type antennae (E-beh. 5/15, hybrid-beh. 8/10, nonresponders-beh 12/16, and E & H-beh. 0/1), and to a lesser degree for the Z-type antennae (E-beh. 2/8, hybrid-beh. 1/5, nonresponders-beh 1/7, and E & H-beh. 0/0).

Discussion

The electrophysiological response profiles of male *O. nubilalis*, *Trichoplusia ni*, and *Ctenopseustis* sp.[6,16,17] among others, are good examples of the phenomenon that in moths the main pheromone component elicits responses from a large spike amplitude cell in an olfactory sensillum, whereas another minor component elicits responses from a small spike amplitude cell in the same hair. Because of this trend, it has been suggested that the spike amplitude, not just the spike frequency, may have an influence on the behavior that is evoked by the activity of these cells[17]. However, the present study clearly demonstrates that it is not a requirement that positive behavioral responses and large action potential amplitude be matched during a response to the behaviorally 'major' component.

Genetic studies involving various crosses and backcrosses of E and Z ECB races have shown that the loci for antennal olfactory cell response and behavioral response are independently inherited[4], and, thus, certain progenies should include some genetically unusual males that possess an antennal type that should not be compatible with the full-flight behavioral responses exhibited by those males. The present study shows that these unusual males do indeed exist. However, there is no obvious explanation for why the distribution of antennal types differs from the 1:2:1 ratio of E:I:Z types found in previous studies. With the particular F_2 progeny produced in this study, there were 8 males of the $Z^EZ^EA^ZA^Z$ type that possessed Z-type antennae but fully responded behaviorally to the E blend. Normally Z males do not respond behaviorally to the E blend. Additionally, there were 15 males of the $Z^EZ^EA^EA^Z$ type that possessed hybrid (intermediate) antennae but fully responded behaviorally to the E blend. Hybrid males normally do not respond behaviorally to the E

blend. Finally, there were 17 males of the $Z^EZ^ZA^EA^E$ type that possessed E-type antennae, but fully responded to the Z blend and were classified as hybrid responders. Normally E males do not respond to the Z blend.

The existence of unusual males that respond behaviorally to a particular pheromone blend regardless of the antennal type provides support for the hypothesis[18] that the sex-linked factor associated with behavioral responses functions is in the central nervous system (CNS). In the CNS processing of moth pheromones, integration of incoming activity from the periphery is complex, involving a number of anatomical structures and neuronal pathways in the brain sensitive to different features of the pheromone mixture[19,20,21,22]. The frequencies of incoming spikes from peripheral cells selectively responsive to different components are the initial elements in this process, and in several species the ratios of such frequencies have been shown to change with blend ratio and to be correlated with behavioral activity[23,24].

Spike size, while apparently not critical for behavioral response, is correlated with the diameter of the neurons' dendrites[25]. A larger neuronal diameter may well be facilitating the presence of more receptor sites specific for the pheromone component to which that cell is tuned and result in a higher sensitivity (higher firing rate) to that component. The relationship may explain why our large-spiking cells fired with higher frequencies than small-spiking cells to a given concentration of pheromone component.

The results from this study show that the CNS systems of E- and hybrid-behavioral-type males can accept and respond to the somewhat altered ratios of spike frequency input that will occur when the 'wrong' antennae are present on their bodies. For instance, the reversals in E and Z receptor cell sensitivity in our Z-antennated unusual males (fig. 6, top left group) compared to normal E-antennated males (fig. 6, top right group) were not sufficiently great as to observably reduce their ability to fly upwind to the E blend in these assays, although the success rate of this type of unusual male was not compared to that of normal E-antennated, E-behavior males. The input ratios of E-cell activity to Z-cell activity in either the Z, E, or hybrid antennal type (top center), would still be predominantly in favor of E cells during flight in the E blend plume in which the E component predominates by 99:1 over the Z. The CNS system of E-behavioral-type males has apparently developed to respond selectively to such E-dominated ratios of E:Z spike frequency ratio input, but not to the predominately Z input of the Z blend, even though for Z-antennated males that input is from the larger peripheral cells.

Similarly, hybrid-behavioral-type males with E antennae (fig. 6, middle right group) should have the antennal cell sensitivities shifted more toward the E pheromone component from the equal E and Z component input of normal hybrid antennae (fig. 6, middle center group). This change of input, however, was not enough to prevent the unusual hybrid males from flying to the Z blend, similar to normal hybrid males. Also, like normal hybrid males, these E-antennated hybrid males did not respond to the E blend, even though the E component input is through the larger peripheral cells with the higher spike size and faster firing rate. Once again the CNS appears to override changes in the incoming frequency ratios generated by reversals in the size of the antennal cells and still responds according to its behavioral type. The results of this study do not mean that the antennae have no effect on behavior, only that their effect on the behavioral outcome in this instance will have been relatively small; these assays were not designed to detect subtle shifts in blend ratio preference that may have occurred in males with unusual antennal inputs.

The electrophysiological recordings from single olfactory sensilla on male antennae correspond well with earlier published data for ECB[4,6], with the exception of the relative size of the spikes elicited from Z9–14:OAc cells. In both the parental races and in the hybrid, a large cell responded to Z9–14:OAc with an amplitude comparable to those elicited by the main pheromone components. The earlier data[6] reported the spike amplitudes of this behavioral antagonist[11] to be very small. The differences between the size of spikes in the Z9–14:OAc cell in the two studies could be due to differences in electrical filtering in the recording equipment, since the signal-to-noise ratio of extracellularly recorded nerve impulses from pheromone receptors can be strongly influenced by the selected filtering range[26]. Regardless of how large these spikes really are, it is clear that these cells remained a relatively constant size in ECB males of all genotypes. Therefore, the genetic determinants of the spike amplitude of these Z9–14:OAc-specific cells do not appear to be linked to those that orchestrate the building of Z11- or E11-OAc-sensitive olfactory cells.

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