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Incomplete electrical isolation of sex-pheromone responsive olfactory receptor neurons from neighboring sensilla

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Abstract

In the long trichoid sensilla on male *Helicoverpa zea* antennae, approximately 40% of the sensilla having a large-spiking olfactory receptor neuron responding to the major pheromone component, (*Z*)-11-hexadecenal, also exhibit small-spiking action potentials that also seem to be responsive to this same compound. In this study, we investigated whether these small-spiking signals are a result of intrusive electrical signals generated from neighboring sensilla. Two methods were used for this study. First, the sensillum was completely covered by the saline-filled recording electrode to physically prevent the sensillum from being contacted by exposure to (*Z*)-11-hexadecenal. In this case, activation of the large-spiking neuron in response to the pheromone component was prevented, whereas the small-spiking activity continued to be influenced by the airborne delivery of the pheromone. In the second method the (*Z*)-11-hexadecenal was applied directly in solution through the cut tip of the sensillum through the recording electrode. In this case only large-spiking activity occurred in response to (*Z*)-11-hexadecenal, with no increase whatsoever in the firing frequency of the small spikes. We conclude that these long trichoid olfactory sensilla are not completely isolated electrically from neighboring sensilla and that small spikes in some recordings originate from large-spiking olfactory receptor neurons (ORNs) in neighboring sensilla.

Keywords: Insect; Sex pheromone; Olfaction; Sensillum; Cut-tip single sensillum recording; Electrical isolation; Lepidoptera; *Helicoverpa zea*

1. Introduction

In insect olfactory systems, unlike those of vertebrates, from one to three olfactory receptor neurons (ORNs) are co-localized within each olfactory trichoid sensillum, surrounded by accessory (auxiliary) cells and the cuticle of the sensillum (see review, Hildebrand and Shepherd, 1997). The dendrites of the ORNs are bathed in an aqueous gel (sensillum lymph) inside the cuticle. Each sensillum can be functionally distinguished according to the physiological response characteristics of the ORNs, due to their response profiles to different odorants. The most widely used methods for sensillar characterization have been the cut-tip single sensillum recording method and the tungsten electrode penetration technique, coupled with stimulation by arrays of odorants (Boeckh, 1962; Kaissling, 1974; Van Der Pers and Den Otter, 1978).

The majority (ca. 70%) of the sexually dimorphic long trichoid sensilla (type-A sensilla) on the antennae of the male moth, *Helicoverpa zea,* house an ORN responding specifically to the female-emitted major pheromone component, (*Z*)-11-hexadecenal (Z11-16:Ald) (Roelofs et al., 1974; Klun et al., 1979; Cossé et al., 1998; Lee et al., 2006a). Type-B sensilla (ca. 10%) have a single odor-responsive ORN that responds only to (*Z*)-9-tetradecenal (Z9-14:Ald). The remaining 20% of long trichoid sensilla (type-C sensilla) contain two odorant-responsive ORNs. One ORN exhibits larger spikes and responds to both the *H. zea* secondary pheromone component (*Z*)-9-hexadecenal (Z9-16:Ald) as well as to Z9-14:Ald. The second, co-localized, ORN in this type of sensillum exhibits smaller spikes and responds to the behavioral antagonists (*Z*)-11-hexadecenyl acetate (Z11-16:OAc) and

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(*Z*)-11-hexadecen-1-ol (Z11-16:OH) (Cossé et al., 1998; Lee et al., 2006a).

The type-A sensillum of *H. zea* had been thought to house only one ORN due to recordings showing only this one large-spiking ORN's responsiveness to Z11-16:Ald, and having its axonal arborization destination occurring 100% of the time in a specific glomerulus (the cumulus) of the male-specific pheromone-related glomerular structure, the macroglomerular complex (MGC) (Cossé et al., 1998; Vickers et al., 1998; Baker et al., 2004; Lee et al., 2006a). However, neuronal cobalt staining of the ORNs of this sensillar type also revealed that about 40% of the staining attempts in these sensilla resulted in a second neuron projecting their axons 100% of the time to a specific glomerulus outside of the MGC, which was named the PCx-1 (Lee et al., 2006a, b).

Besides the Z11-16:Ald-responsive large-spiking ORN, extremely small action potentials also had been observed occasionally during recordings from type-A sensilla of heliothine moths (Lee et al., 2006a, b; Berg et al., 1998). Transmission electron micrographs showed that the vast majority of *H. zea* male long trichoids house one very large dendrite and one very small dendrite (not shown). The small-spiking signals from type-A sensilla were considered to be generated from the PCx-targeting neuron (Lee, 2006), on the basis of reports that dendrite size is correlated with spike size in male *Ostrinia nubilalis* (Hansson et al., 1994) and in male *Antheraea polyphemus, Bombyx mori* (Kaissling et al., 1978; Keil, 1984a; Meng et al., 1989; Kumar and Keil, 1996). However, this correlation may not hold for male *Antheraea pernyi* (Meng et al., 1989; Kumar and Keil, 1996).

To attempt to find a specific ligand that might elicit these smaller action potentials, an array of ca. 60 compounds was tested in recordings on this sensillum in a high-throughput screening regime (Lee, 2006), but these tests could not confirm any active odorants that stimulated what we thought should be a small-spiking cell. Curiously, only the volatiles known to activate the large-spiking ORN, Z11-16:Ald and its hydrocarbon analog, 1,(*Z*)-12-heptadecadiene (Grant et al., 1989; Lee, 2006), were effective in reliably also evoking spike trains of very small amplitude.

The septate junctions present in the apical regions between a dendrite's accessory cells and epithelial cells form a diffusion barrier among three regions, the sensillum lymph space, the subcuticular space, and the hemolymph space. The very tight contact of the apical membrane of the tormogen cells with the cuticle in *A. polyphemus* trichoid sensillum had been shown to electrically and morphologically isolate the sensillar lymph space of one sensillum from those of neighboring sensilla (Keil, 1984b; de Kramer, 1985; Keil and Steinbrecht, 1987). Hence, according to the weight of evidence from the above prior reports, we interpreted the small-spiking action potentials found in type-A sensilla recordings as originating from within the sensillum connected to the recording electrode, from the cell having the small dendrite (Lee, 2006; Lee et al., 2006a).

Nevertheless, we subsequently felt we could not exclude the possibility that the small-spiking action potentials observed from the cut-tip sensillum recordings might be generated from neighboring sensilla, taking into consideration the coincidental spiking episodes between large- and small-spiking cells and their similar dose-response profiles of response to both Z11-16:Ald and 1,(*Z*)-12-heptadecadiene (Lee, 2006). Also, there had been a previously published acknowledgement of possible "electrical interference" from olfactory neurons in neighboring sensilla, even when using the cut-tip technique, due to a purportedly weak electrical isolation between sensilla (Nagai, 1983; de Kramer, 1985; Kaissling, 1995), and this led us to investigate these small-spiking signals further. In our study, two opposite approaches were taken to verify whether or not the small-spiking action potentials originated from neighboring sensilla: stimulation of only the neighboring sensilla and not those in the recording sensillum (Kaissling, 1995; Stange and Kaissling, 1995); and stimulation of cells only in the recording sensillum and not those in the neighboring sensilla (Van den Berg and Ziegelberger, 1991; Pophof, 2002).

2. Materials and methods

2.1. Insects

The *H. zea* colony was maintained on a 16:8 L:D photoperiod at 25 °C, 40–50% RH. Larvae were reared on a modified pinto-bean diet (Shorey and Hale, 1965). Males and females were separated in the pupal stage and housed in separate growth chambers in different rooms. The moths used in this experiment were males from 1 to 3 days old.

2.2. Chemicals

The major sex-pheromone component of *H. zea*, Z11-16:Ald, was purchased from Bedoukian Research Inc. (Danbury, Connecticut, USA; purities >98% verified by gas chromatography), and dissolved in hexane solution to a concentration of 100 μg/μl in preparation for solubilization in saline solution for direct application of pheromone through the recording electrode. For both the conventional and sensillum-covering airborne stimulation by puffing into the airstream, 10 μl of a hexane solution of Z11-16:Ald (1 μg/μl) was loaded onto a 0.7 × 2.5 cm filter paper strip and the solvent allowed to evaporate before placing the strip into a 146 mm-long Pasteur pipette odor cartridge. The preparation and storage of cartridges was as described by Baker et al. (2004). Four other pheromone-related chemicals, Z9-16:Ald, Z11-16:OAc, and Z9-14:Ald, were utilized to identify the sensillum type using airborne stimulations as per the protocol of Cossé et al. (1998). Cartridges for each compound were prepared from the compounds (neat) stored in our laboratory with the same protocol as used for Z11-16:Ald.

2.3. Conventional procedure for ORN recordings using the cut-tip technique

The activities of ORNs in a sensillum were recorded using the cut-sensillum technique (Kaissling, 1974; Van der Pers and Den Otter, 1978; Cossé et al., 1998). The recording method was the same as reported previously (Baker et al., 2004; Lee et al., 2006a), except that in order to eliminate noise generated by muscle movement when using a whole-body preparation, these recordings were performed using an excised antenna cut at the base of the flagellum approximately 1–2 mm from the pedicel (c.f., Todd et al., 1992; Hansson and Löfstedt, 1987). The base of the excised antenna was inserted into a saline-solution-filled indifferent glass electrode having a sufficiently wide opening for receiving the antenna's base. The recording electrode was connected to a Universal AC/DC probe assembly with pre-amplifier (Syntech, Hilversum, The Netherlands). Neuronal activity was amplified by the built-in amplifier of a micromanipulator system (INR-05, Syntech), digitally processed by IDAC 4 (Syntech). Spike frequency was analyzed using a spike-counting program (Autospike32, Syntech) after data acquisition.

Air-puffs through the odor cartridges for odorant stimulation were generated with a flow-controller device (SFC-2, Syntech) using 100-ms pulses at 20 ml/s flow rate. Odorants in the puffs issued through a small inlet into an air stream flowing through a 7.6 mm ID glass tube, whose outlet was positioned 2.5 cm from the antenna. The air stream flowed continuously over the antenna at a rate of 8 ml/s during the stimulation. Once the odorant cartridge tip was inserted at right angles into the air stream 85 mm upstream from the outlet of the continuous-air stream tube, a stimulation was triggered 2 s after the new trace appeared on the computer monitor in order to eliminate subjective selection of the stimulating moment. Each encountered sensillum was examined using the conventional cut-tip sensillum technique to identify the sensillum type before the sensillum covering or the direct application in saline (see below).

2.4. Stimulation of only neighboring sensilla by covering the recording sensillum

External application by puffing air through the odorant cartridge will possibly stimulate not only the ORNs in the sensillum from which recordings are made, but also perhaps those residing in multiple neighboring sensilla across a broad area of the antenna. By completely covering the recording sensillum with the electrode, we felt we could potentially record the neuronal activities of ORNs in neighboring sensilla in response to puffs of Z11-16:Ald without stimulating the ORNs within the electrode-covered sensillum. If we found that small spikes could be observed in recordings, but not large spikes, we could conclude that these small spikes must be coming from ORNs residing in neighboring sensilla.

Only the sensilla located the most laterally and closest to the scale region were used for this covering experiment to minimize the number of neighboring sensilla (Fig. 1). In order to cover the targeted sensillum with the recording electrode, we used the micromanipulator to slip the electrode over the sensillum and keep pushing the electrode towards the sensillum base until the antenna visibly moved backward slightly, indicating that the antennal cuticle was being pushed by the tip of the electrode (Fig. 3).

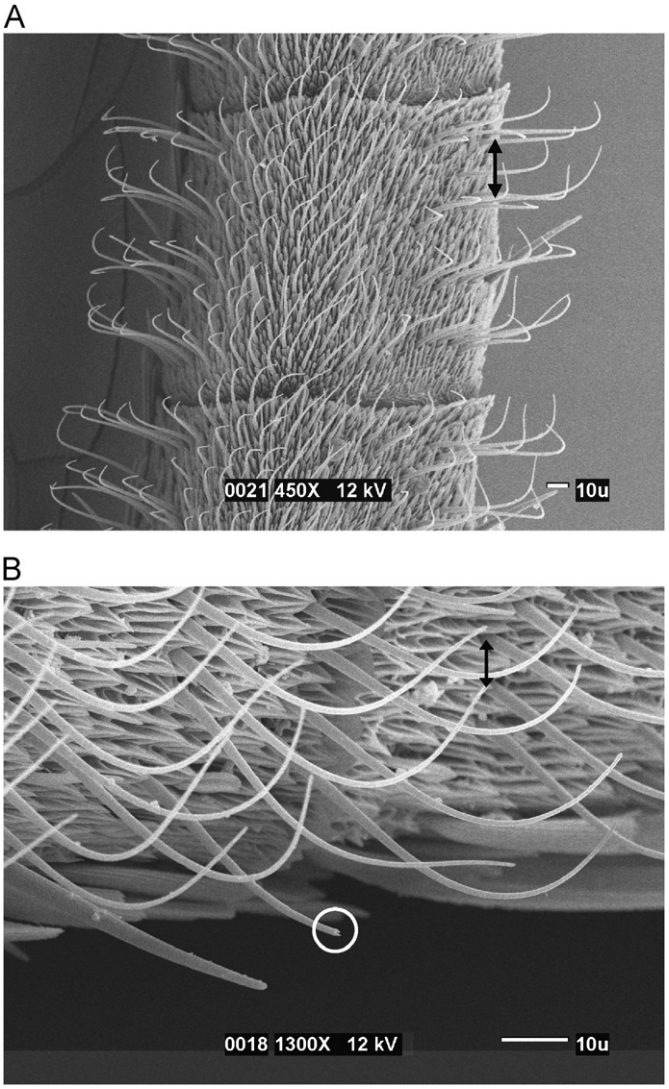

Fig. 1. Scanning electron micrograph of *H. zea* male antenna flagellomere. (A) The male-specific longer trichoid sensilla are arrayed in four rows in one flagellomere segment, and observed more prominently on the equatorial region where they are closer to the scales. The shorter sensilla trichodea, on the other hand, are found in the medial region with irregular distribution. The average distance between two rows (up-and-down arrow) in a segment is 26.9 μm (S.E. = ±0.7, $N = 32$). (B) Higher magnification image representing arrangement of long trichoid sensilla in each row. The average distance between two sensilla (measured at the base portion of each sensillum, up-and-down arrow) in the same row is 7.4 μm (S.E. = ±0.2, $N = 53$). For the covered sensillum recordings, we used the most laterally located sensilla close to the scales. The white circle indicates the cut tip of the sensillum prepared for single sensillum recording. Scale bars 10 μm.

The airborne puffing procedure was then conducted in the same way as during the conventional tip recording method employing airborne delivery of the odorants.

As described above, spike frequencies were determined using a spike-counting program (Autospike32, Syntech) after data acquisition. The number of spikes during 1 s of pre-stimulation was subtracted from the number of spikes occurring during 1 s after stimulation, and so the frequency increment per second was considered as the activity change in response to the airborne stimulation. Only hexane was loaded on the filter paper of the "blank" cartridge. The control stimulation procedure was carried out using the conventional cut-sensillum technique, in which the tip of the recording electrode was only allowed to contact the distal end of the cut sensillum.

2.5. Stimulation of neurons only within the recording sensillum by direct application of Z11-16:Ald

In contrast to the sensillum-covering experiment, we attempted to try to stimulate only the neurons residing in one sensillum that came into contact with the recording electrode, using direct application of Z11-16:Ald in solution in the electrode (Fig. 5); this method should exclude ORNs in the neighboring sensilla from being stimulated. If the small action potentials recorded from the contacted sensillum during airborne puffing were coming from ORNs residing in neighboring sensilla, then direct application should not be able to generate small spikes. To put it another way, strong activity from small spikes using direct application from the electrode would be evidence for the existence of activity of a small-spiking ORN from within the recording sensillum. We needed to do this in case the small-dendritic-diameter neuron in type-A sensilla was contributing small spikes that could be confused with additional spikes of the exact same size originating from outside the sensillum we were recording from.

For these experiments, the Z11-16:Ald was solubilized in saline following the method used by Pophof (2002) with minor modification. We applied 2.5 μl of the Z11-16:Ald hexane solution (100 μg/μl) to the glass vial (2 ml) and left it at room temperature for 30 min in order to evaporate the hexane. DMSO-dissolved saline (dimethyl sulfoxide; EMS, Fort Washington, PA, USA; purity >99.9%) (0.01%) was added to the vial, and then was incubated for 1 day at room temperature in a rotator. The incubated solution was transferred to a 1.5 ml plastic microcentrifuge tube, and then centrifuged (Micromas, Thermo IEC, Needham Heights, MA, USA) at 10,000 rpm for 1 min. A portion of the solution was taken from the bottom of the 900 μl volume and the remnant, including the surface portion, was discarded, which possibly contained undissolved Z11-16:Ald. The Z11-16:Ald-solubilized saline was transferred to a glass vial and stored in a refrigerator (4 °C) until used for the experiment. The numbers of large and small spikes were counted for 1 s after the spike frequencies were established following contact with the Z11-16:Ald-containing saline in the recording electrode. Saline with the 0.01% DMSO but having no Z11-16:Ald dissolved in it was used for the control. The spike activities of direct saline solution applications were compared with those during 1 s following the airborne stimulation of Z11-16:Ald.

2.6. Statistical analysis

Frequencies of large and small spikes were analyzed independently to determine whether there were any differences among different stimulation conditions. Pairwise comparisons were carried out between two of three different conditions for each large- or small-spike frequency data using a least square difference (LSD) *t*-test.

3. Results

From 161 type-A standard cut-tip sensillum recordings using airborne stimulation, which include data acquired for other independent experimental projects, we found that ca. 40% (64 sensilla) exhibited small-spiking action potentials along with the Z11-16:Ald-responsive large-spiking action potentials. The spike sizes of these smaller action potentials were not consistent, but the majority (57 out of 64) were less than one-third the amplitude of the large action potentials visible in the same spike trains. The spike amplitudes in the remaining seven recordings could occasionally be as large as one-half those of the large spikes (Fig. 2).

3.1. Stimulation of only neighboring sensilla by covering the recording sensillum

Seventeen sensilla exhibiting spontaneous small-spiking activity were used for airborne puffing in the covered-sensillum experiment (Fig. 3). Before covering, using only the conventional contact between the tip of the sensillum and the tip of the recording electrode, both small- and large-action potentials were observed to increase in frequency in 12 of these sensilla when Z11-16:Ald was puffed. However, the remaining five sensilla exhibited only large-spiking ORNs that increased in spike frequency when Z11-16:Ald was presented. The 12 sensilla exhibiting Z11-16:Ald-responsive small- and large-spiking activity were then used further for completely covering these sensilla with the recording electrode.

When these 12 sensilla were completely covered by the electrode, airborne presentation of Z11-16:Ald failed to elicit large-spiking action potentials in all cases (Fig. 4). Only the frequency of small spikes in these recordings increased in response to Z11-16:Ald to levels that were not significantly different from the frequencies seen during the conventional, uncovered tip recordings (Fig. 4). This result indicates that the increased small-spiking activity in response to Z11-16:Ald must have come from ORNs in other sensilla, because lack of excitation of the large-spiking ORN showed that the sensillum from which we

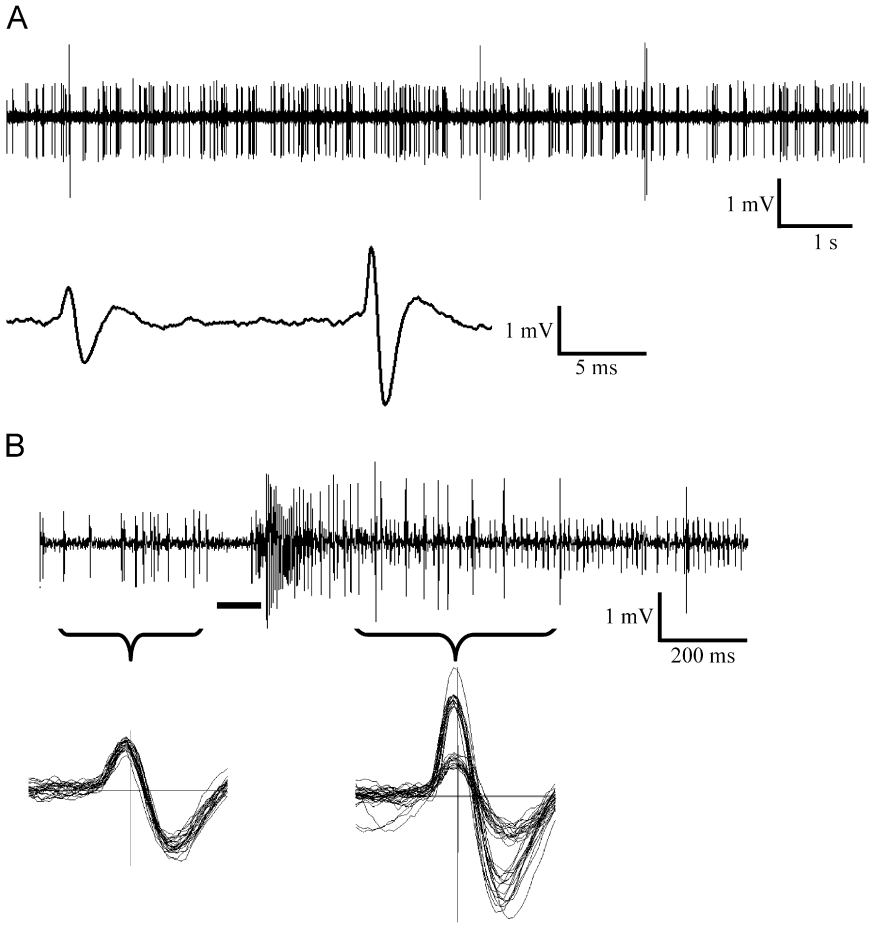

Fig. 2. Large- and small-amplitude spike trains from a type-A sensillum, illustrating one of the largest-amplitude recordings of the small-spiking action potentials. (A) Tracing of small- and large-amplitude action potentials recorded from a type-A sensillum. Lower panel shows different waveforms in expanded time scale. (B) Spike train during a recording from a type-A sensillum. Both small- and large-spiking action potentials occur in response to stimulation with Z11-16:Ald in a 100 ms pulse (horizontal bar). Superimposed wave forms of small- and large-spiking action potentials are illustrated for the period represented with brackets.

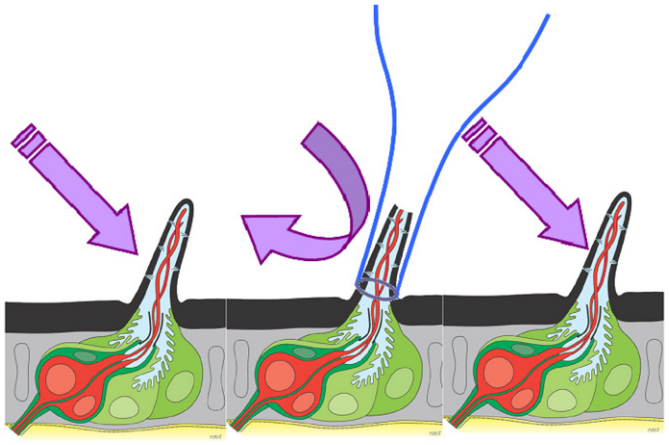

Fig. 3. Schematic diagram illustrating the method for covering a sensillum with the recording electrode and the presentation of the pheromone odorant. Complete covering prevents the sensillum from being exposed to the airborne stimulant while the neighboring sensilla are exposed. The illustration was modified from Steinbrecht (1999).

were recording had been successfully occluded by the saline electrode covering the sensillum.

For one sensillum that exhibited small and large spikes in response to conventional puffs of Z11-16:Ald we attempted to eliminate the neuronal activities of the five nearest-neighboring sensilla by application of 0.5 M cobalt–lysine solution (Hansson et al., 1992) until no neuronal activities were observed there in response to the series of external stimulations. After inactivating only the neurons residing in the closest two sensilla (possibly through toxicity effects) in each of the two neighboring rows, the small-spiking activities were still not different from the activities before the deletion of neuronal activity in the neurons in these four sensilla. However, after inactivating the neurons in the very next sensillum in the same row on the medial side of the recording sensillum, the smaller spiking activities completely disappeared (data not shown). In this instance at least, the small-spiking activities were shown to have been generated from within the very

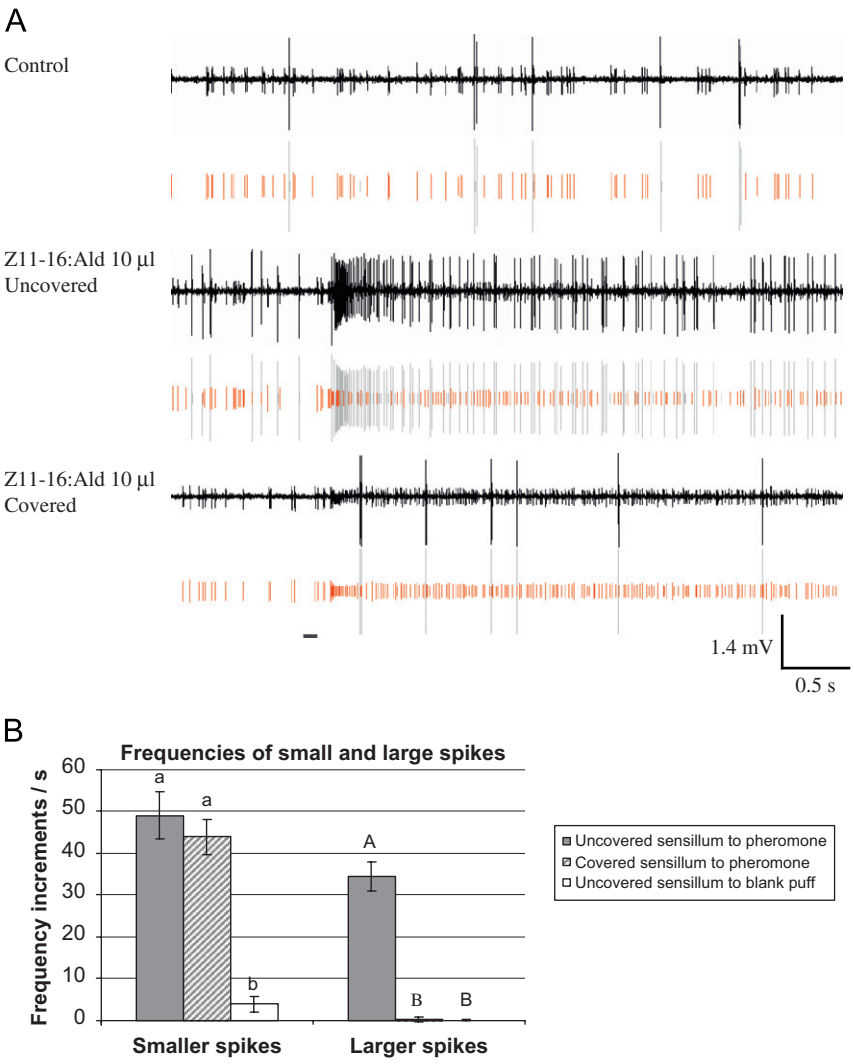

Fig. 4. Comparative activities of the small and large action potentials recorded using the conventional cut-tip single sensillum recording compared to recordings made by covering the sensillum. (A) Spike-train response profiles of a type-A sensillum to 10 µg Z11-16:Ald airborne stimulation. Covering the sensillum with the recording electrode prevents the sensillum cuticle from being exposed to Z11-16:Ald, and so the large action potentials were not elicited. Nevertheless, the covering did not affect the frequency of small spikes that were evoked in response to airborne puffs, and at a level that was similar to that which was elicited during conventional recording. Lower panels in each stimulation trace are simplified spike trains with spike filtering in order to visualize the small-spiking action potentials clearly. Horizontal bar denotes 100 ms stimulation. (B) Mean values (±S.E., $N = 12$) of spike frequency increase for 1 s following airborne stimulation with 10 µg Z11-16:Ald. Large- and small-spike frequencies were analyzed independently. The lower-case letters denote statistical comparisons between small-spike frequencies and the upper case letters are for the larger spike statistical comparisons. Different letters indicate significant differences according to a *t*-test with least square difference correction for pairwise comparisons ($\alpha < 0.05$).

next sensillum in the same row. From measurements on three antennae using a scanning electron microscope, we found that the average distance between two sensilla in the same row (7.4 ± 0.2 µm S.E., $N = 53$) is shorter than the distance between two closest sensilla in different rows (26.9 ± 0.7 µm S.E., $N = 32$) (Fig. 1).

For the five sensilla in which Z11-16:Ald elicited only large action potentials and failed to elicit small ones, we used the standard cut-tip technique to examine three of these sensilla further using airborne delivery of other pheromone-related chemicals such as Z9-16:Ald, Z11-16:OAc, and Z9-14:Ald. These are the odorants to which ORNs in type-C sensilla of *H. zea* are responsive. The activities of small action potentials in recordings from all three of these large-spiking-only sensilla in response to the Z11-16:Ald increased markedly after stimulation with these other compounds. Interestingly, when the neighboring sensilla in the same row and next to these three sensilla were then recorded from, they were identified as type-C sensilla (data not shown) due to their large spikes in response to these compounds. These activities were apparently picked up as small spikes from within the neighboring type-A sensilla.

3.2. Stimulation of neurons only within the recording sensillum by direct application of Z11-16:Ald

Direct application with Z11-16:Ald in saline solution was attempted for seven type-A sensilla that exhibited

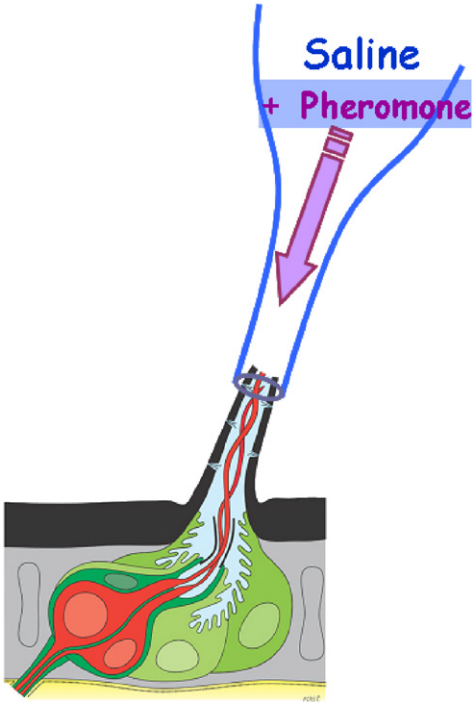

Fig. 5. Schematic diagram illustrating the method of direct delivery of Z11-16:Ald dissolved in saline through the cut-tip opening of the sensillum. This illustration was modified from Steinbrecht (1999).

Z11-16:Ald-responsive small- and large-spike activity using standard puffing (Figs. 5 and 6). Stimulation of ORNs within these sensilla using Z11-16:Ald in solution through the recording electrode increased the frequency of only the large-spiking action potentials. The frequency of small action potentials was not significantly different from those seen during spontaneous firing (Fig. 6). Thus, direct application of Z11-16:Ald through the recording electrode failed to affect the frequency of small spikes in the recordings, unlike what was seen during airborne application using puffs of Z11-16:Ald.

Exposure of the ORNs to the dissolved Z11-16:Ald for several minutes caused the large-spiking ORNs to become adapted, and to be no longer responsive to airborne stimulation using the Z11-16:Ald cartridge. On the other hand, the small-spiking ORN activity was not adapted, as seen in the increases in small-spike activity in response to subsequent airborne stimulation with Z11-16:Ald (Fig. 6A). This appears to be further evidence that the small spikes are not generated from within the sensillum from which we were attempting to record.

4. Discussion

We undertook this study in the process of trying to determine to which odorant molecules, if any, the so-called silent neuron in type-A *H. zea* sensilla might be responsive. This neuron is co-localized with the Z11-16:Ald-responsive, large-spiking ORN (Lee et al., 2006a, b) that routinely projects to a particular glomerulus, the PCx1, in the posterior complex that lies immediately posterior to the MGC in *H. zea* antennal lobes (Lee et al., 2006a, b). We needed to be able to ascertain whether the very small spikes seen in the recordings when Z11-16:Ald or its analog was presented were due to the silent neuron or due to neighboring ORNs residing in other sensilla. Our results and those of Lee (2006), in which this neuron was challenged with an array of prospective general and pheromonal odorants, continue to support the classification of this neuron as being silent, or unresponsive. These results also are informative in showing that, with the cut-tip single sensillum recording technique, there can be instances in which electrical activity from ORNs in neighboring sensilla can potentially confound interpretations of recordings from purportedly electrically isolated sensilla.

The ORNs within sensilla have usually been thought to be isolated functionally and electrically from neighboring ORNs (Keil, 1984b; de Kramer, 1985). However, de Kramer (1985) reported that electrical insulation is not perfect between adjacent sensilla. The resistance between adjacent sensilla in the same longitudinal row of male *A. polyphemus* is as high as about 2000 MΩ, and, although signals from ORNs in neighboring sensilla were "very much attenuated", they were in fact detected (de Kramer, 1985).

Kaissling (1995) also alluded to possible electrical interference from neighboring sensilla, which he said could be confirmed by completely covering the sensillum with the recording electrode. However, in the intervening years, the possibility of incomplete electrical insulation between neighboring sensilla had not been scrutinized. This issue becomes important in using the cut-sensillum recording technique to characterize, for instance, the odorant affinities of secondary, co-compartmentalized secondary neurons such as those that reside in heliothine moth type-A sensilla (Lee et al., 2006a, b).

In our current study, the increase in the frequency of small spikes followed by airborne puffing of Z11-16:Ald was not affected by whole-sensillum covering with the recording electrode compared to the uncovered conventional method. Nor was the small-spike frequency altered from its spontaneous background level by direct application of Z11-16:Ald through the electrode. These results taken together strongly indicate that the small-spiking action potentials seen in our cut-tip recordings from the type-A sensillum are not elicited by the PCx1-targeting secondary neuron that has a small-diameter dendrite, but rather they originate from one or more large-spiking, Z11-16:Ald-responsive ORNs residing in neighboring sensilla.

Thus, we now suggest that the secondary neurons in these type-A sensilla that arborize in the PCx1 are not responsive to Z11-16:Ald or its hydrocarbon analog (Lee, 2006), and we have avoided making an erroneous conclusion about what, if any, odorant-specific information this glomerulus receives. We still have found no odorant that stimulates this small dendritic diameter neuron and do not

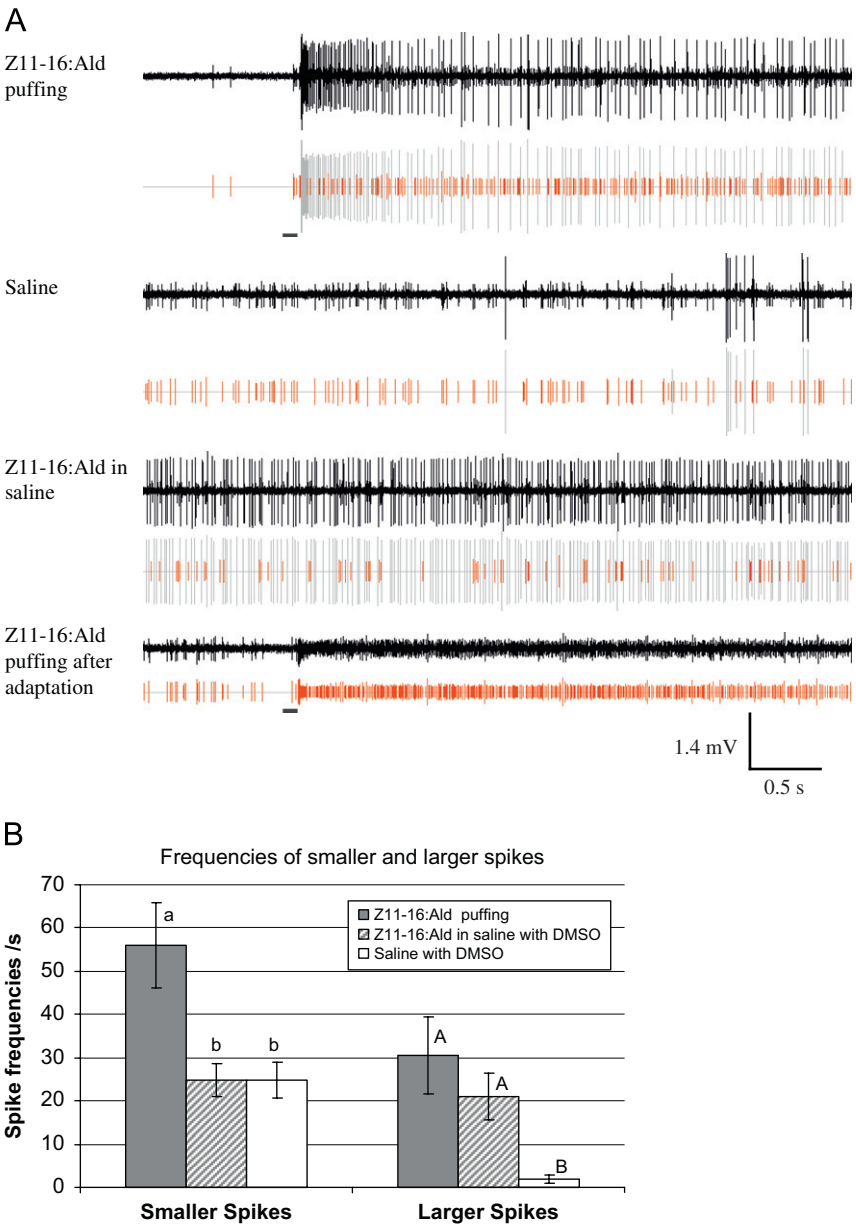

Fig. 6. Activities of the small and large action potentials that were recorded using conventional cut-tip single sensillum recording with airborne puffs of Z11-16:Ald, compared to direct stimulation with this solubilized compound in saline delivered through the recording electrode. (A) Spike trains recorded from a type-A sensillum in response to these stimulation regimes. Direct delivery of the solubilized Z11-16:Ald stimulated the large-spiking neuron, but small-spiking responses were not evoked with this direct application. After about 2 min of exposure to the stimulant, the large-spiking ORN became adapted, but the small spikes are still highly responsive to airborne Z11-16:Ald stimulation, which indicates that the neuron generating the small spikes is not located in the sensillum housing the large-spiking ORN. Lower panels in spike trace are simplified by using spike filtering in order to visualize the small-spiking action potentials clearly. Horizontal bars denote 100 ms stimulations. (B) Mean values ($\pm$S.E., $N = 7$) of spike frequency increase during 1 s of exposure to the airborne stimulation with Z11-16:Ald as well as during direct application. Large- and small-spike frequencies were analyzed independently. The lower-case letters denote analyses of the small-spike data, and the upper cases are those related to the large spikes. Different letters indicate significant differences according to a *t*-test with least square difference correction for pairwise comparisons ($\alpha < 0.05$). Small-spiking activity in response to direct delivery of Z11-16:Ald was not significantly different from the application of saline only.

know the nature of the odorant information, if any, that leads through the PCx-1.

Our findings provide a note of caution in possible studies in which ORN dendrite diameters would be attempted to be correlated with spike size despite the elegant work of Hansson et al. (1994) and Kumar and Keil (1996). It would be important, as we have done here, to attempt to validate first that any very small spikes that ride just above the recording noise level, as seen in our recordings, are generated from within the sensillum from which recordings are made when using the cut-sensillum technique. Our findings strongly indicate that in type-A *H. zea* sensilla these small spikes originate from ORNs in neighboring sensilla.

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