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Working Range of Stimulus Flux Transduction Determines Dendrite Size and Relative Number of Pheromone Component Receptor Neurons in Moths

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Abstract

We are proposing that the "relative" abundances of the differently tuned pheromone-component-responsive olfactory receptor neurons (ORNs) on insect antennae are not a result of natural selection working to maximize absolute sensitivity to individual pheromone components. Rather, relative abundances are a result of specifically tuned sensillum-plus-ORN units having been selected to accurately transduce and report to the antennal lobe the maximal ranges of molecular flux imparted by each pheromone component in every plume strand. To not reach saturating stimulus flux levels from the most concentrated plume strands of a pheromone blend, the dendritic surface area of the ORN type that is tuned to the most abundant component of a pheromone blend is increased in dendritic diameter in order to express a greater number of major pheromone component–specific odorant receptors. The increased ability of these enlarged dendrite, major component–tuned ORNs to accurately report very high flux of its component results in a larger working range of stimulus flux able to be accurately transduced by that type of ORN. However, the larger dendrite size and possibly other high-flux adjustments in titers of pheromone-binding proteins and degrading enzymes cause a decrease in absolute sensitivity to lower flux levels of the major component in lower concentration strands of the pheromone blend. In order to restore the ability of the whole-antenna major pheromone component–specific channel to accurately report to its glomerulus the abundance of the major component in lower concentration strands, the number of major component ORNs over the entire antenna is adjusted upward, creating a greater proportion of major component–tuned ORNs than those tuned to minor components. Pheromone blend balance reported by the whole-antennal major and minor component channels in low plume–flux strands is now restored, and the relative fluxes of the 2 components occurring in both low- and high-flux strands are thereby accurately reported to the component-specific glomeruli. Thus, we suggest that the 2 phenomena, dendrite size and relative numbers of differentially tuned ORNs are linked, and both are related to wide disparities in molecular flux ranges occurring for the more abundant and less abundant components in the pheromone blend plume strands.

Key words: channel capacity, dendrite size, insect pheromones, odorant flux ranges, odorant receptor abundance, olfactory receptor neurons, olfactory sensilla

Introduction

Over decades of insect pheromone research, it is clear that the superabundance of pheromone component–tuned olfactory receptor neurons (ORNs) on male antennae exists as a result of selection for exquisite sensitivity in detecting and reporting pheromone components in the environment by optimizing the environmental signal-to-noise ratio. Tens of thousands of long hair-like, trichoid sensilla aid in the gathering of pheromone-related information from the air and in amplifying the signal due to the ORNs' convergence onto only a few projection interneurons in the antennal lobe of the brain (Boeckh J and Boeckh V 1979; Boeckh and Selsam 1984).

For moths, we have learned also that it is the pheromone "blend" to which males have the lowest threshold for initiating upwind flight behavior and to which they sustain upwind flight all the way to the source (Baker et al. 1981; Linn et al. 1986, 1987). Thus, fidelity of blend ratio reporting from the ORNs to the antennal lobe is of primary importance to pheromone communication.

Given the exquisite behavioral sensitivity that has been selected for in the huge numbers of male moth ORNs, a puzzling and perplexing question has persisted over the decades. Why, in several large insect groups such as noctuid

moths, and even in bark beetles, is the greatest "proportion" of ORNs tuned to the most abundant pheromone component in a species' sex pheromone blend? This is illustrated in North American heliothine moths (Figure 1), the bark beetle species *Ips pini* (Figure 2), and the turnip moth, *Agrotis segetum* (Figure 3), in which for all these species, component-specific ORNs are housed individually in their own separate sensilla. The geographic correlation between the proportion of major and minor pheromone component–tuned ORNs and the relative abundances of different components in emitted pheromone blends of *I. pini* and *A. segetum* are particularly striking (Figures 2 and 3).

There has been a tacit explanation for the greater abundance of ORNs tuned to the major component of moth pheromones: they must be there to increase sensitivity of detection of pheromone. However, neuronal detection of a single component does not produce upwind flight at the lowest emission rates (Linn et al. 1986, 1987); the composition of the complete blend communicated across the ensemble of differentially tuned component-specific ORNs is what causes upwind flight at the lowest behavioral thresholds. With the usual reasoning concerning detection of single components, it would make more sense that the "minor" components would be the ones requiring a greater number of ORNs tuned to them in order to provide a greater detection sensitivity. However, it is the ORNs tuned to the less abundant components that are the ones that are less abundant themselves. This would seem to make the minor components "more" difficult, not less, to detect! Thus, the "heightened sensitivity" reason given for devoting greater numbers of ORNs to the most abundant component in a blend has never made sense.

A second unresolved question exists for other insect families such as tortricid, pyralid, saturniid, and yponomeutid moths in which 2 or more pheromone component–tuned ORNs are cocompartmentalized in the same sensillum. Why does the ORN tuned to the most abundant pheromone component in the blend produce a larger amplitude action potential than the ORN tuned to the minor component and apparently have a larger diameter dendrite? Is this somehow related to sensitivity of detection, and if so, why would the dendrite having the greater surface area for accommodating multitudes of odorant receptors (ORs) be the one that is tuned to the most abundant component and not to the trace component? We propose that the answer to this question helps resolve the above conundrum concerning relative

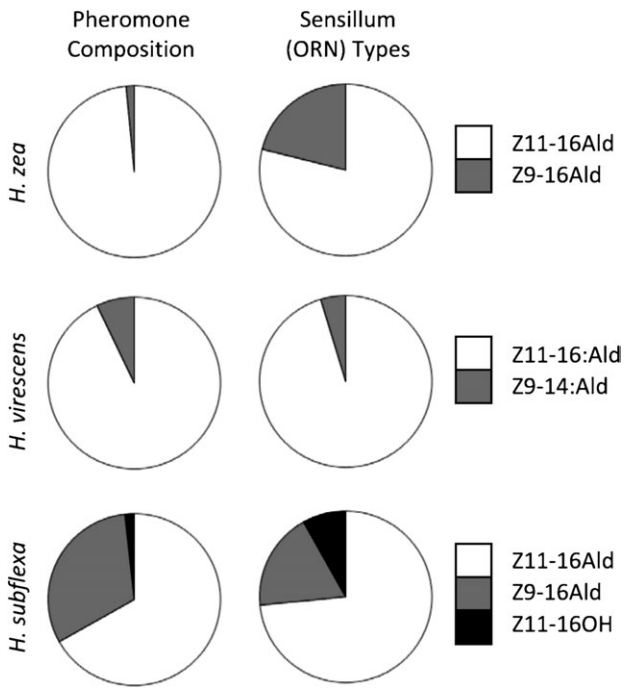

Figure 1 Percentage of sensillum types (right column) with ORNs tuned to the indicated sex pheromone components of 3 North American heliothine species, *Heliothis virescens*, *Heliothis subflexa*, and *Helicoverpa zea*. Percentage of sex pheromone components emitted by females in each species' blend is depicted in left column. All 3 species use Z11:16 Ald as the major pheromone component (Roelofs et al. 1974; Tumlinson et al. 1975; Klun et al. 1979; Teal et al. 1981) but differ in the use of minor components (Heath et al. 1990; Pope et al. 1982, 1984). A majority of trichoid sensilla have ORNs tuned to the major component Z11:16 Ald with sensilla having ORNs responding to minor components being relatively uncommon (Cossé et al. 1998; Baker et al. 2004).

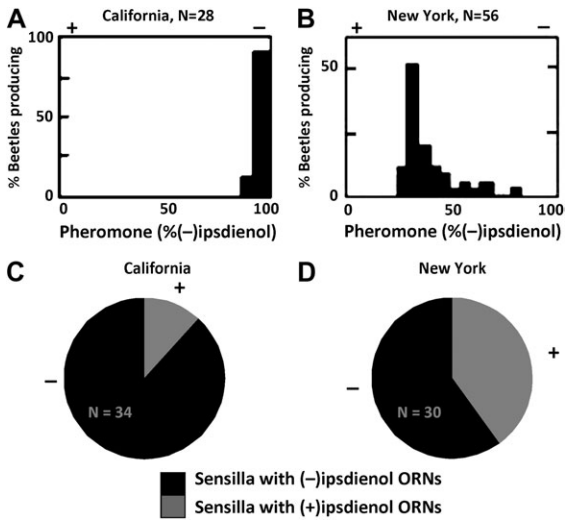

Figure 2 Eastern and western *Ips pini* bark beetle populations differ in their use of the enantiomers of the monoterpene ipsdienol in their aggregation pheromone, with western populations using almost exclusively (–)-ipsdienol and eastern populations blends of 50–70% (+)-ipsdienol (Birch et al. 1980; Lanier et al. 1980; Miller et al. 1989). Depicted here are recent descriptions of the individual male pheromone production profiles in the (**A**) California and (**B**) New York populations (Domingue and Teale 2007). Surveys in the 2 populations found that in (**C**) western populations, more than 90% of pheromone component–sensitive sensilla contained ORNs tuned to (–)-ipsdienol versus (+)-ipsidenol, whereas in eastern populations (**D**), there were more balanced numbers of the sensillum types containing ORNs tuned to (–)-ipsdienol or (+)-ipsidenol (Mustaparta et al. 1980, 1985). Mustaparta et al. (1980, 1985) speculated that perhaps these patterns of *Ips* bark beetle sensillar type distributions assisted in allowing each species to have greater sensitivity in detecting the major pheromone components.

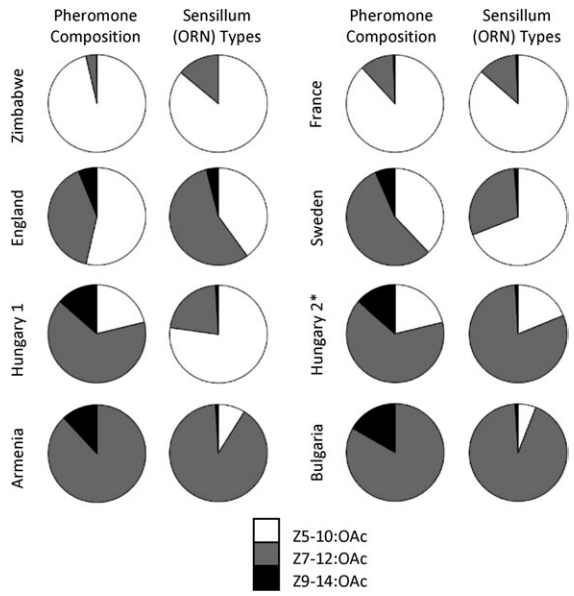

Figure 3 The turnip moth, *Agrotis segetum* has 3 homologous pheromone components, but 4 confirmed pheromone components in total (Wu et al. 1995): Z5-10:OAc, Z7-12:OAc, and Z9-14:OAc (Bestmann et al. 1978; Arn et al. 1980; Tóth et al. 1980). There is considerable variation in the relative amounts of these compounds found in pheromone glands (Löfstedt et al. 1986; Hansson et al. 1990) and in the most effective blends for field trapping throughout Europe, Asia, and Africa (Arn et al. 1983; Tóth et al. 1992; Wu et al. 1999). Here, we show the gland composition ratios measured in the above studies corrected according to the disparate volatilities of the different chain lengths of the compounds (Liljefors et al. 1985). To the right of each pheromone composition, we depict the proportions of ORNs on the antennae that were found to be tuned to each of the 3 components (Wu et al. 1999). In Hungary, there were 2 sensillar distribution phenotypes, which were reported separately, but pheromone gland extracts were pooled, leaving no indication as to whether there is similar polymorphism for this trait. Generally, the results over all the populations show a correlation between the relative pheromone component abundance and the proportions of ORNs tuned to particular pheromone components.

ORN abundances. We suggest that the answer again "does not" have to do with increasing the ORN's sensitivity. Rather, we propose that dendrite size is due to the adjustments that have had to be made by major pheromone component–sensitive sensilla-ORN units to accurately transduce the more widely differing ranges of molecular flux occurring in the major versus the minor components in plume strands of their species' pheromone blend.

The stimulus flux transduction ranges of ORNs

The functional unit on the antennae of male moths for acquiring, detecting, and processing flux is the *sensillum trichodeum*. These organules (Lawrence 1966) have been designed as flux detectors and not concentration measurers (Kaissling 1998) due to the need of the moth to behaviorally respond as rapidly as possible to individual pheromone strands and the pockets of clean air between strands that comprise the fine-grained structure of an airborne pheromone plume (Baker and Haynes 1987; Baker 1990; Kaissling 1990, 1998; Vickers and Baker 1994). Flux involves a time component, for example, "molecules per second," and flux detectors accentuate the onset and offset of the signal. For reporting changes in flux, a flux detector does not allow the system to come into equilibrium but rather concentrates the molecules at onset and rarifies them at offset (Kaissling 1998). This is accomplished by a combination of pheromone-binding proteins (PBPs) in the perireceptor lymph that quickly remove the pheromone molecules (called "pheromone deactivation" by Kaissling 1972, 2009) and by pheromone-degrading enzymes (PDEs) (Ishida and Leal 2005), without which ORNs stimulated with a short pulse of the component to which they are tuned may fire tonically for many seconds (Syed et al. 2006). The coordination between ORs, PBPs, and PDEs allows for rapid registration and reporting of widely varying flux in each newly encountered strand of pheromone and the clean air pockets between strands.

The molecules of all the pheromone blend components contained in each plume strand rush over the antenna relative to the insect's airspeed as the insect flies upwind. At a given airspeed, the more concentrated strands create higher fluxes of all the components. However, it is the most abundant component in the blend that creates the highest range of molecular flux from strand to strand, or strand to clean air pocket, at any airspeed. The major component is often present in 10 or 100 times the amount of the minor components. The blend strands can vary from very high to very low concentration, depending on the degree to which they have been shredded by microturbulence (Murlis 1986). Therefore, the upwind flight system that depends on precise pheromone component blend ratios must be able to receive accurate reports, via high-fidelity molecular flux transduction by the ORNs, about the molecular abundance "ratios" of the pheromone components in both high- and low-concentration strands.

Adjustments in flux transduction ranges at the sensillum level: species having larger spiking ORNs tuned to the major pheromone component

In tortricid species such as *Argyrotaenia velutinana* (redbanded leafroller) and *Epiphyas postvittana* (light brown apple moth), yponomeutids in the genus *Yponomeuta*, silkmoths in the genera *Antheraea* and *Bombyx*, sphingids such as *Manduca sexta*, and pyralids in the genus *Ostrinia*, 2 ORNs responding to the most important components in the sex pheromone blend are housed together in the same trichoid sensillum (O'Connell 1975; Van Der Pers and Den Otter 1978; Kaissling 1979; Hansson et al. 1987, 1994; Akers and O'Connell 1988, 1991; Kaissling et al. 1989; Meng et al. 1989; Löfstedt et al. 1990; Kumar and Keil 1996). In male moths from the genera in which the ratio of abundance of the major to the minor components is very high (e.g., >4:1), 1 of the 2 ORNs exhibits a large spike size

and responds to the major pheromone component in the 2-component blend and the second ORN exhibits a smaller spike size and responds to the less abundant component. In *M. sexta*, the blend ratio is approximately 2:1, and the spike sizes of the cocompartmentalized ORNs are rarely discernably different, but when they are, the ORN tuned to the major component has the larger spike size (Kaissling et al. 1989).

Most tellingly, in the European corn borer, *Ostrinia nubilalis*, there are 2 races that use reversed ratios of (*Z*)-11-tetradecenyl acetate (Z11-14:OAc) to (*E*)-11-tetradecenyl acetate (E11-14:OAc) as their respective pheromones, with the "Z" race using a 97:3 and the "E-race" using a 1:99, ratio of these 2 components (Roelofs et al. 1987). Hansson et al. (1994) demonstrated with meticulous experimentation that ORN spike size is related to the diameter of the dendrites (Figure 4). In both the E and the Z strains of *O. nubilalis*, the larger spike-sized ORN of the 2 colocalized ORNs in a sensillum responds to the major component. These authors suggested that dendrites with " a larger such area, housing more ion channels, could give rise to an action potential with higher amplitude" (Hansson et al. 1994). In the F1 hybrids between the 2 strains, the 2 ORNs acquire more similar spike sizes in concordance with the intermediate 35:65 Z11-/E11-14:OAc blend ratio emitted by hybrid females (Roelofs et al. 1987). In hybrids, the sudden shift to more similar spike sizes of the Z11- and E11-responding ORNs is accomplished by the parental strain's larger diameter ORN apparently shrinking and the smaller diameter ORN growing (Hansson et al. 1994). We would suggest that the intermediate and nearly equal ORN dendrite sizes of hybrids may optimize the transduction of the nearly equal molecular flux ranges of the 2 components when hybrid males fly to hybrid females.

That the spike size (dendrite diameter) in *O. nubilalis* is an important characteristic apart from ORN tuning profile (OR expression) comes from additional information gleaned from additional hybridization studies (Olsson et al. 2010). Hansson et al. (1987) had found that spike size was inherited autosomally and thus independently from the sex-linked behavioral pheromone preferences of male moths. This finding suggests that spike size is an independent trait upon which selection may operate to produce an optimal phenotype for a particular population; it thus clearly is not simply an arbitrary pleiotropic effect of other pheromone-related traits. Olsson et al. (2010), however, when revisiting this system, showed that although spike size was again largely autosomally determined, there was also a smaller degree of sex-linked influence of the trait, concomitant with sex-linked determination of behavioral upwind flight to the blends. This additional sex-linked ORN spike size effect may be representative of the importance of this trait, which we would suggest is related to the different ranges of flux transduction needed by major versus minor component–tuned ORNs.

We assume in our current paper that the differences in spike sizes of the colocalized ORNs in the other families

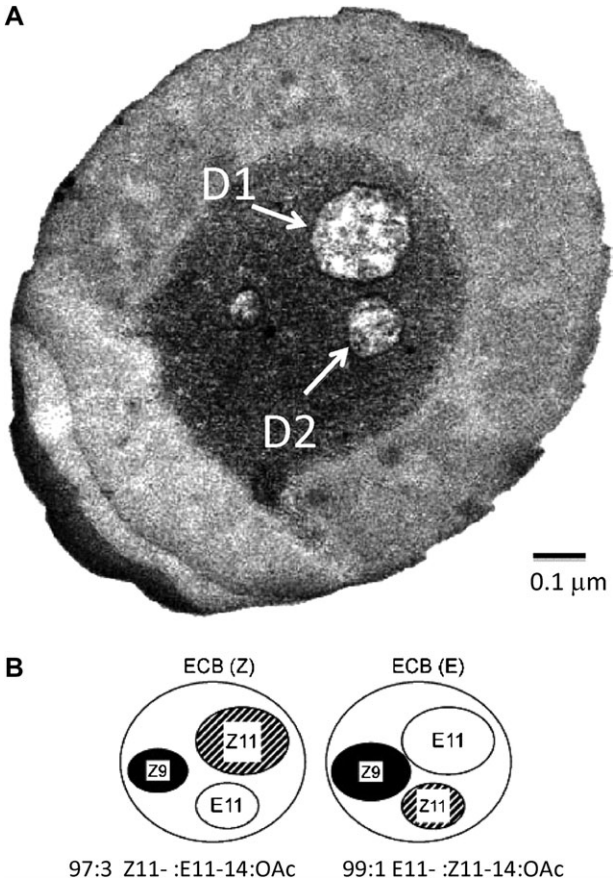

Figure 4 (**A**) Cross-section transmission electron micrograph of a trichoid sensillum from an *Ostrinia nubilalis* E-strain male antenna showing a large diameter dendrite (D1) plus an intermediate-sized (D2) and small dendrite (Domingue MJ, Lee SG, Baker TC, unpublished data). (**B**) Depicted here (according to the spike sizes measured in Domingue et al. 2006 and Domingue et al. 2007, respectively), the dendrite of the large spiking ORN in Z-strain ECB (left) tuned to Z11-14:OAc has the larger dendrite corresponding to D1 in A above, whereas the ORN tuned to E11-14:OAc has the depicted smaller relative dendrite diameter corresponding to D2 above. Also in this sensillum, there is the smallest spiking ORN tuned to a behavioral antagonist, Z9-14:OAc, with a dendrite illustrated as having the smallest diameter. In the E-strain (right schematic) the situation is reversed, with the ORN tuned to E11-14:OAc having the larger diameter dendrite (Hansson et al. 1987, 1994; Domingue et al. 2007) with its size depicted relative to the other 2 dendrite sizes. Although a recent study (Olsson et al. 2010) has suggested more variation exists in spike size of hybrid male ORNs (not illustrated here) than previously recognized, the average spike sizes of parental type ORNs tuned to the major component are more consistent and are larger than the average spike size of ORNs tuned to the minor component.

mentioned above are similarly correlated with dendrite diameter. Apart from the Hansson et al. (1994) study, elegant work by Kumar and Keil (1996) demonstrated a similar neuroanatomical correspondence between spike size and dendrite diameter. They used an unusual technique they discovered of monitoring the pheromone component–induced selective damage to the microtubular cytoskeletons of the dendrites of 1 of the 2 ORN types that are cocompartmentalized in the sensilla of *Bombyx mori*, *Ant. polyphemus*,

and *Ant. pernyi*. The antennae were exposed to high concentrations of 1 of the 2 sex pheromone components of each species, and the sensilla were then examined via transmission electron microscopy to determine whether ORNs with large versus small dendrites had been selectively stimulated and tagged morphologically by the visible disruption of their microtubules (Kumar and Keil 1996). Meng et al. (1989) had previously shown on the antennae of *Ant. polyphemus* and *Ant. pernyi*, in which long trichoid hairs predominate and make up 85% of all trichoid hairs, that large spikes were elicited in each species by its major pheromone component and small spikes by the minor component.

The results of Kumar and Keil (1996) corresponded to the previous electrophysiological findings of Meng et al. (1989) for *Ant. polyphemus* and those of Kaissling (1979) for *B. mori* but not for *Ant. pernyi*. In *B. mori* and *Ant. polyphemus*, exposure to the major pheromone component disrupted the microtubules of the larger diameter ORN and exposure to the minor component disrupted the smaller diameter ORNs' microtubules. The reverse was true for *Ant. pernyi* (Kumar and Keil 1996). Thus, in 3 of 4 lepidopteran species and 5 of 6 pheromone communication systems (including both of the *Ostrinia* strains and the F1 hybrids), there is neuroanatomical support that large spikes and small spikes correspond to large and small dendrite diameters and responsiveness to major and minor pheromone components, respectively (Hansson et al. 1994; Kumar and Keil 1996).

The case of *Ant. pernyi* may provide an interesting exception to our hypothesis and should be examined further. Also, even when large spiking ORNs can be found to be the ones responding to minor components, as in the case of experimentally generated, odd F2 hybrids of *O. nubilalis* E and Z strains (Cossé et al. 1995), normal upwind flight behavior to normal parental blends can occur. Nevertheless, it is not known in such cases whether the flux "ranges" that can be transduced might have been diminished by these reversals in dendrite diameters, and whether behavioral responsiveness across wide ranges of emission rates may have been compromised.

A larger surface area on the larger spiking ORN will be able to express greater numbers of the OR tuned to the major component in order to bind and process the molecules having the greatest abundance and report this relative abundance under the high-flux short time frames dictated by the plume strands encountered several times per second in nature (Baker and Haynes 1989). Accommodating the more abundant pheromone component in a strand should require that there be more ORs on the dendrite of an ORN tuned to the major component than there are ORs tuned to the minor component on its specifically responding ORN. Such a relationship can be seen in transmission electron micrographs in which sensory neuron membrane proteins (SNMPs) associated with pheromone-sensitive ORs (Benton et al. 2007) have been stained in situ (Figure 5) (Rogers et al. 2001). SNMPs are known to be required for pheromone sensitivity in insects (Jin et al. 2008). The larger diameter dendrite in *Ant. polyphemus* clearly has a higher density of SNMPs on its dendritic surface than does the smaller dendrite (Figure 5).

When the working range of component-specific flux transduction in a sensillum is exceeded

In *A. segetum*, in-flight arrestment of males' upwind progress occurs in plumes in which the emission rate of the total blend is too high; the working range of pheromone component–specific flux transduction in one type of ORN has been exceeded, whereas the flux range in the other 2 types of ORNs has not (Hansson and Baker 1991). At these behaviorally excessive emission rates in a wind tunnel pheromone plume, the plume-strand flux of the highest emission rate component, (*Z*)-5-decenyl acetate (Z5-10:OAc), exceeds saturating levels for its type of ORN, and these ORNs become adapted and cease firing in response to the strands. However, the ORNs tuned to the minor components (*Z*)-7-dodecenyl acetate and (*Z*)-9-tetradecenyl acetate (Z7-12:OAc and Z9-14:OAc, respectively) receive flux from their components in these same strands that is well within their working ranges and continue to fire (Figure 6). The ratio of components in the pheromone plume strands has not changed, but male upwind flight behavior becomes arrested because the blend ratio reported to the 3 different antennal lobe glomeruli (Hansson and Baker 1991) is now unbalanced (Baker 2008) and skewed toward the 2 minor components. The previously behaviorally optimal approximately 1:1:1 firing frequency is now dominated by excitation from the still-reporting ORNs tuned to the 2 less abundant components due to the relative silence from the ORNs tuned to Z5:10:OAc (Figure 6) (Hansson and Baker 1991; Todd and Baker 1999).

Range of component-specific flux transduction of an ORN and its absolute sensitivity

We are suggesting that it is at the sensillum-ORN organule level at which the working ranges of component-specific flux can be adjusted (Kaissling 2009) by increasing or decreasing the number of ORs on a dendrite. One way to do this would be to increase or decrease the OR density by expressing greater or lesser numbers of ORs per membrane area. In Kaissling's (2009) model of the kinetic events resulting in pheromone molecule transduction that occur in the sensillum, it was stated that "large numbers of receptor molecules are required for the wide range of stimulus intensities covered by the dose-response curves of the receptor potential." For the upper range of flux transduction, if OR density is already at its maximum, then increasing the size of the dendrite will result in an increased surface area and allow the expression of more ORs. Such dendrites will be able to accurately transduce the greater flux from the more abundant pheromone components while still reporting flux from lower concentration plume strands. Dendrite size might also

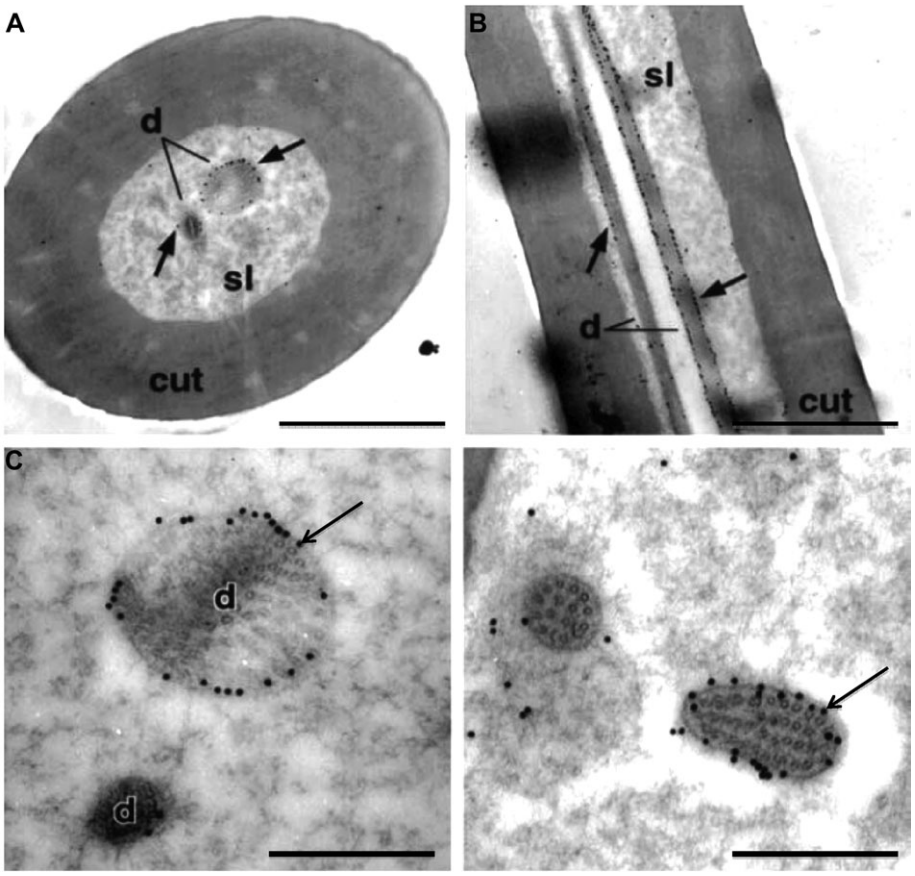

Figure 5 Transmission electron micrographs of SNMP-1 sites (dark black dots) on the dendrites of male *Antheraea polyphemus* in trichoid sensilla, indicating a higher density of SNMP-1-coupled ORs (arrows) on the larger diameter dendrite than on the smaller dendrite. (**A**) Cross-section of sensillum; (**B**) Longitudinal section of sensillum; (**C**) Two cross-sections of pairs of dendrites. d = dendrite, sl = sensillar lymph (Rogers et al. 2001). Scale bars: A, 1.25 μm; B, 2.5 μm; C, 0.5 μm.

be altered through changes in its length, but in ORNs cocompartmentalized in the same sensillum, there will be a constraint on length, and so for a major component–tuned ORN, increasing its diameter would seem to be the only option. The sensillar organule does not operate as a flux transducer by means of ORNs and ORs alone, however. The concentrations of perireceptor factors such as PBPs can also be adjusted to meet the challenges of wide-ranging molecular flux in pheromone plume strands (Kaissling 2009).

In Kaissling's (2009) model, equation (52) demonstrates the relationship between the need for higher numbers of ORs (R_{tot}) so that the stimulus saturation level (U_{sat}) is raised sufficiently high that the broadest optimal working range of stimulus flux transduction can occur. PBP concentrations also contribute to modifying the saturation level (Kaissling 2009). This insight into flux transduction working ranges is consistent with the observation that the ratios of different pheromone component–specific forms of PBPs in the long trichoids of *Ant. polyphemus* and *Ant. pernyi* (Maida et al. 2003) are roughly directly correlated with the relative abundances of the pheromone components (C16 Acetate: C16 Aldehyde: C14 Acetate) in the blends of each species. Maida et al. (2003) found that PBPs 1, 2, and 3 that bind the 3 components above, respectively, are present in *Ant. polyphemus* sensilla in the ratio 70:1:30 (pheromone blend is 90:10:0) and in *Ant. pernyi*, they are present at 50:50:1 (pheromone blend ratio estimated at 20:100:40 by Bestmann et al. 1978).

Considering that higher levels of PBPs for higher strand flux will be needed, as well as higher concentrations of PDEs, in order to more quickly clear the lymph of pheromone after large flux, it is understandable that having a larger dendrite with more ORs does not necessarily mean that the absolute sensitivity of the ORN in such a wide working range sensillum will have been increased. Calculations of Kaissling's (2009) illustrate that without the protection from PDE degradation afforded by the pheromone being bound to PBPs, the sensitivity of the system would go down significantly. PBPs also turn into a "scavenging" form (Kaissling 1998) that help remove pheromone from the system after it has interacted with the ORs (Kaissling 2009).

The existence of several forms of PBP specific to major and minor pheromone components in some species is consistent with independent flux transduction regulation for cocompartmentalized ORNs having greater or lesser numbers of

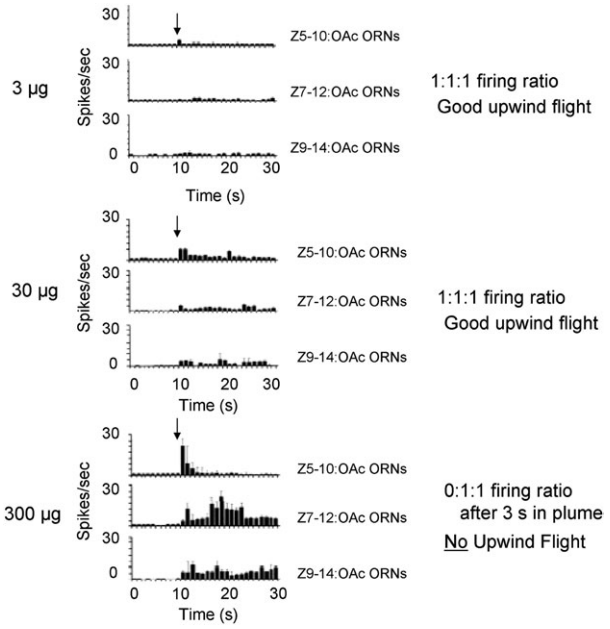

Figure 6 Responses of 3 types of differently tuned ORNs on the antennae of *Agrotis segetum* in a wind tunnel in response to natural point source plumes from a rubber septum emitting the 3-component sex pheromone blend of this species: a 10 to 5 to 0.25 ratio of Z5-10-OAc to Z7-12:OAc to Z9-14:OAc, respectively. Plumes emitting the same blend ratio but at 3 different septum loadings were wafted over the preparations. The 3 types of ORNs recorded from were: 1) those tuned to Z5-10:OAc (black histogram bars are each 1-s averaged responses from 33 ORNs exposed to the plume); 2) Z7-12:OAc-tuned ORNs (bars are 1-s averages from 7 ORNs); and 3) the relatively rare Z9-14:OAc-tuned ORNs (bars are 1-s averages from 2 ORNs). None of the neurons responded to any other components than the component they are tuned to. The tracings show the responses during a 10-s prestimulus period, and then for 20 s after the preparation was placed in the plume (black arrows), 1.5 downwind of the septum. The firing rates increased at the 10 s mark (immediately after placement into the plume was allowed to flow over the preparation) in each graph. At the low and mid range source dosages (3 and 30 µg), each of the 3 types of neurons responded to their individual component in the plume for the entire 20 s of exposure to the complete blend plume. The 3 components forming the blend were reported as an approximately 1:1:1 ratio of spike frequency for the 3 ORN types at both the 3 and 30 µg loadings. Because this blend at these 2 dosages resulted in optimal upwind flight to the source, this 1:1:1 firing ratio would thus form the neuronal representation of the 3-component pheromone blend to the antennal lobe of the brain. However, the highest dosage (300 µg) of this same 3-component blend, which caused arrestment of upwind flight before males reached the source in the upwind flight assays, caused the Z5-10:OAc-tuned ORNs on the antennae first to briefly fire excessively and create and 8:1:1 spike frequency ratio with the other 2 ORN types and then to adapt after only several seconds of plume-exposure and to cease firing entirely. The blend ratio reported to the antennal lobe would now be 0:1:1 with no reporting from the Z5-10:OAc ORNs, with the ORNs tuned to Z7-12:OAc and Z9-14:OAc still continuing to accurately report the higher fluxes of these components in the plume for the full 20-s period. From Todd and Baker (1999). Adapted from Hansson and Baker (1991).

ORs present on larger versus smaller diameter dendrites. Independent up- or down modulations in absolute sensitivity of the ORNs can occur, based on the concentrations of the different PBPs and perhaps different PDEs. In *Ant. polyphemus*, one such independently regulated PDE would be specific for the acetate (Vogt et al. 1985; Maida et al. 1995, 2003) and the other for the aldehyde component (Maida et al. 2003).

A lack of increase in absolute sensitivity with larger spiking ORNs can be seen in the ORN recordings of Akers and O'Connell (1988, 1991) of *Arg. velutinana* (Figure 7) using the 2 sex pheromone components of this species that have equivalent vapor pressures. A larger spiking ORN is tuned to the most abundant, "major" component, Z11-14:OAc, and a cocompartmentalized smaller spiking ORN is tuned to the minor component, E11-14:OAc (O'Connell 1975). When the natural female-emitted blend of 92% Z11-14:OAc and 8% E11-14:OAc was puffed over the sensilla, each pair of Z11- and E11-14:OAc-tuned ORN in each sensillum responded with an approximately 3:1 firing ratio of the Z11- to E11-tuned ORNs. If the ORNs had equal levels of absolute sensitivity to their ligands, there should have been approximately a 9:1 spike frequency ratio. The 3:1 firing ratio was registered across a wide range of high and low total action potential frequencies. These results mean that each sensillum allows the small spiking E11-14:OAc-tuned ORN to be more sensitive to its ligand than the larger spiking Z11-14:OAc-tuned ORN is to its ligand when pheromone is encountered as in nature, a simultaneous arrival of the 2 components on the sensillum (Figure 7) (Akers and O'Connell 1988, 1991; Todd and Baker 1999). One uncertainty with these results is that despite the virtually identical vapor pressures of the 2 pheromone components, it is not known whether their emission rates from the odor cartridges were proportional to their loadings in the cartridge and therefore equal.

When 2 pheromone components in a species' blend have different molecular weights or functional groups, then comparisons of firing ratios (or ORN sensitivity) of large and small spiking ORNs must be performed with measured amounts of emitted compounds issuing from odor cartridges and not the loaded amounts. In such cases as in *Helicoverpa zea*, a smaller spiking ORN in "Type C" sensilla was shown to have a higher absolute sensitivity to its behavioral antagonist-related ligands, (*Z*)-11-hexadecenyl acetate (Z11-16:OAc) and (*Z*)-11-hexadecen-1-ol (Z11-16:OH), than did the larger spiking ORN in that same sensillum to its minor pheromone component ligand, (*Z*)-9-hexadecenal (Z9-16:Ald) (Figure 8) (Cossé et al. 1998). Thus, larger spike size again here appears to not be associated with an increase in absolute sensitivity, but it is associated with a greater working range of flux transduction than that of the smaller spiking higher sensitivity ORN (see Figure 8).

When 2 differentially tuned ORNs are not cocompartmentalized (cf. in noctuids), relative spike sizes of ORNs (and dendrite diameters) cannot usually be determined due to differences in electrophysiological recording conditions from sensillum to sensillum and the difficulty in morphologically tagging and later locating a physiologically characterized ORN and measuring its dendrite. There is a clear correspondence in some species, however, between the lengths of

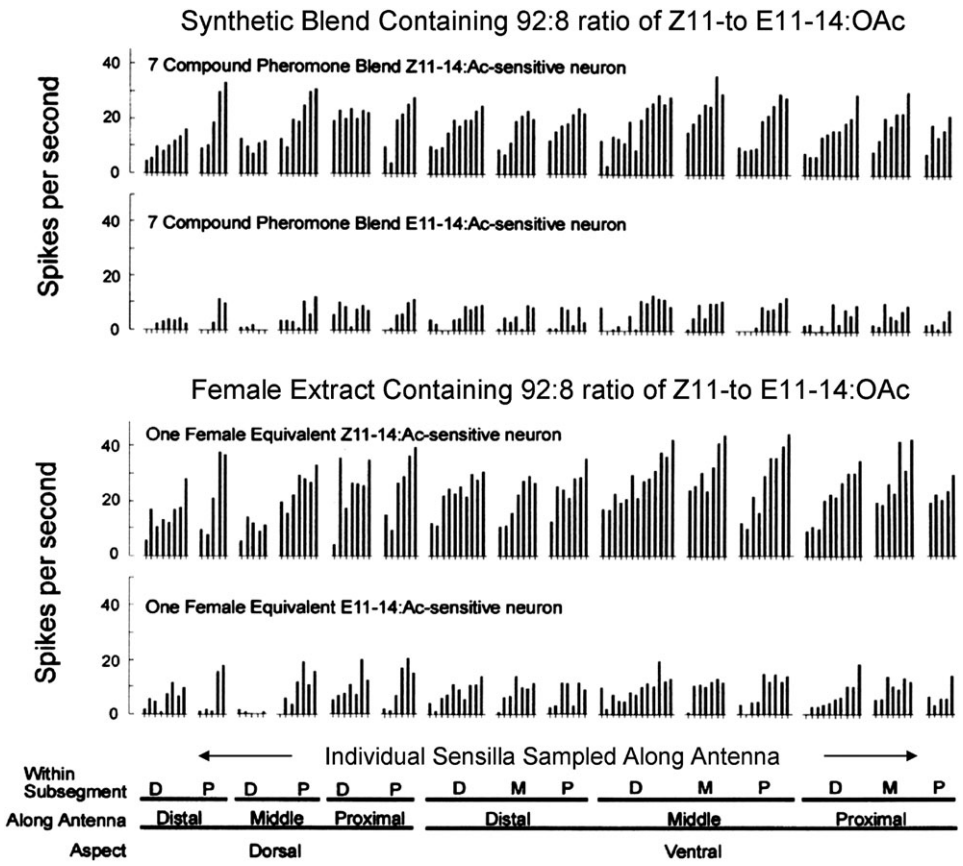

Figure 7 Sensillum-by-sensillum reporting by large spiking "Z11-14:Ac-sensitive" ORNs and smaller spiking "E11-14:Ac-sensitive" ORNs in response to either synthetic (top) or natural gland-extract blends (bottom) of redbanded leafroller pheromone containing a 92:8 ratio of Z11-14:OAc to E11-14:OAc. Note that each sensillum pair of Z11- and E11-sensitive-ORNs reports approximately a 3:1 ratio of spike frequencies even though the actual Z11-/E11-14:OAc abundance is 9:1. This indicates that the smaller spiking ORN is more sensitive to its component in the blend than the larger spiking ORN is to its component. "D" and "P" denote more distal (D) and proximal (P) positions of the sensilla sampled on each antennal subsegment (Todd and Baker 1999; adapted from Akers and O'Connell 1991).

sensilla (and presumably the dendrites within) and the relative abundances of the pheromone components in the blend. In North American heliothine moths, ORNs tuned to the minor component are more commonly found in shorter trichoids and those tuned to the major component predominate in the longer trichoids (Baker et al. 2004; Lee 2006). In *Trichoplusia ni*, the ORNs tuned to the trace component, Z9-14:OAc, were found in sensilla that were recognizably shorter and thinner under the light microscope (Domingue et al. 2009) than the sensilla housing ORNs tuned to the major component, Z7-12:OAc.

Sensillum pores and working ranges of pheromone component–specific flux transduction

We should expect that for sensilla having differently tuned, noncocompartmentalized ORNs, those that house ORNs tuned to major components should have higher densities of pores on their sensillar surface in order to accommodate the entry into the sensillum lumen of the greater flux of molecules to be transported to the dendrites. There is but one example in the literature from physiologically and morphologically characterized olfactory sensilla in which differences in pore densities have been documented. O'Connell et al. 1983 noted that trichoid sensilla of *T. ni* housing ORNs responding to the major pheromone component Z7-12:OAc had a pore density of 15 pores/μm^2, whereas sensilla housing ORNs that were later found to be tuned to a minor component Z7-14:OAc (Grant and O'Connell 1986; Grant et al. 1998) had a density of 7 pores/μm^2. The density of pores for the major component that has a high emission rate of 5 ng/min from females (Haynes and Hunt 1990) is thus more than double that of the rarer Z7-14:OAc-tuned sensilla that must allow entry and transport of this low emission rate minor component, issued at a rate of only 0.05 ng/min from females (Haynes and Hunt 1990).

Although this example of relative pore density with *T. ni* is the only a one we know of for pheromone component–tuned trichoid sensilla, we expect that many systems in which ORNs that report the abundance of different pheromone components and are housed in separate sensillum types might have such a pattern. For general odorant–tuned ORNs that reside in single-walled basiconic sensilla, the much higher density of pores on these structures (Figure 9;

Steinbrecht 1999) is consistent with the need for greater capacity to allow the higher molecular flux occurring from these high emission rate compounds to swiftly and with little impediment enter the sensillum lumen. General odorants from leaves, flowers, fruits, and manure are often emitted into the environment at tens of micrograms per hour per odorant source (cf. Loughrin et al. 1994; Schmelz et al. 2003), rates that are easily 10 000 times greater than from sex pheromone-emitting female moths. In addition to their higher pore density than pheromone-sensitive trichoid sensilla,

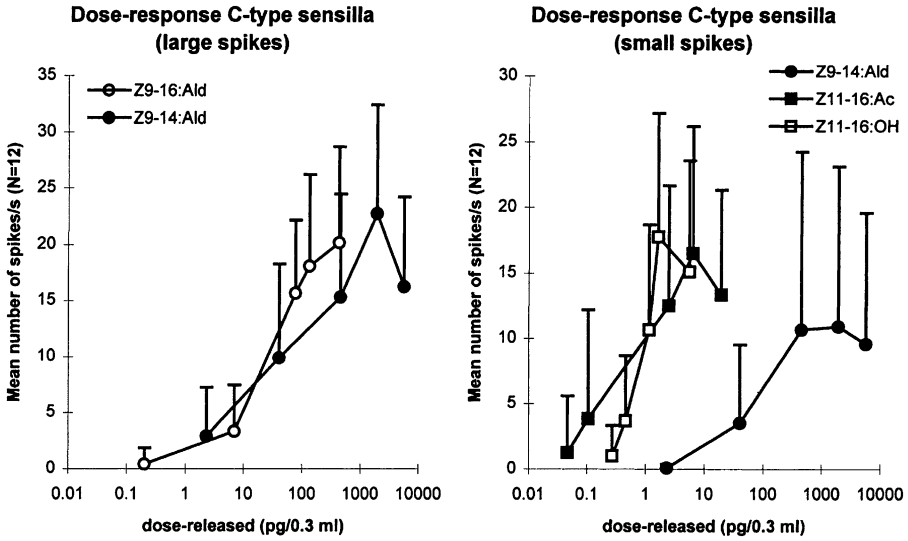

Figure 8 Dose-response profiles of cocompartmentalized large and small spiking ORNs in *Helicoverpa zea*. The large spiking ORN in this type of sensillum (left) responds to both Z9-16:Ald (the minor sex pheromone component) and Z9-14:Ald. The smaller spiking ORN responds to 3 different behaviorally antagonistic compounds: Z9-14:Ald, Z11-16:OH, and Z11-16:OAc but is more sensitive to the latter 2 compounds. This smaller spiking ORN is significantly more sensitive to these 2 compounds than is the larger spiking ORN to its ligand, Z9-16:Ald (Cossé et al. 1998). One should note that the smaller spiking ORN reached its saturation level at an emission (flux) rate nearly 1000 times lower than the emission rate at which the larger spiking ORN reached its saturation level. The amount of pheromone component emitted from an odor cartridge was measured by collecting from 100 to 5000 0.3-ms puffs from the cartridge in a dry-ice-cooled glass melting point tube, rinsing the tube with 20 μg of hexane, and then injecting a few micrograms of solution onto GC along with an internal standard and quantifying the peak heights compared with the height of the known amount of internal standard.

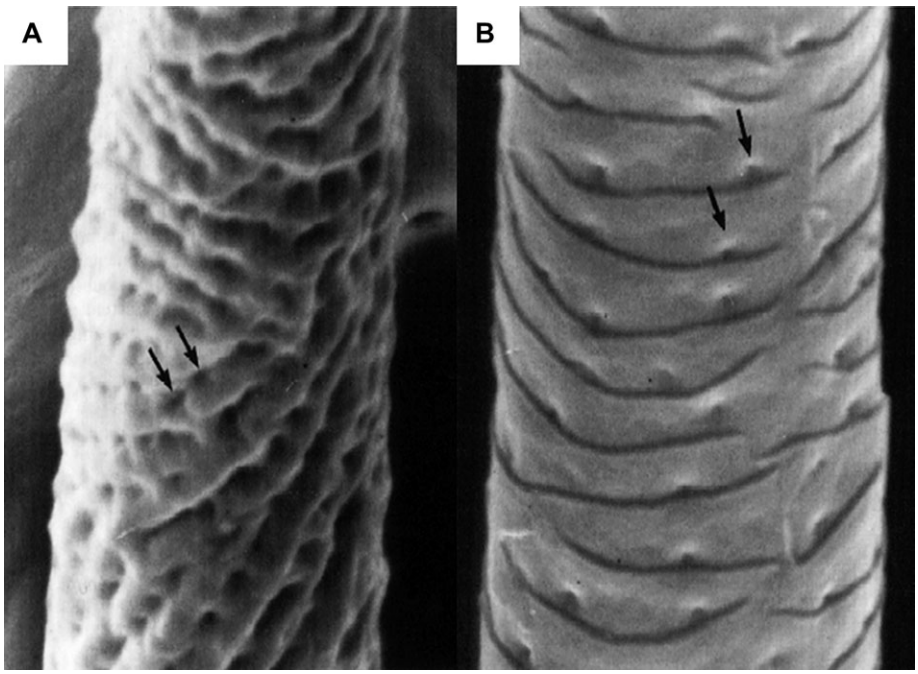

Figure 9 Scanning electron micrographs of: (**A**) a single-wall basiconic sensillum from *Bombyx mori* showing a superabundance of pores (arrows); and (**B**) a trichoid sensillum from *B. mori* showing a more modest density of pores (arrows) across the cuticle. Figure A is 39 000× and B is 40 000× magnification. From Steinbrecht (1999).

the dendrites of the ORNs residing in basiconic sensilla are highly branched, endowing each basiconic ORN with a larger dendritic surface area for receiving these large fluxes from the environment than the unbranched dendrites of pheromone component–tuned ORNs in trichoid sensilla (Figure 10; Rogers et al. 2001). Sometimes the branching is so profuse that the branches occupy nearly the entire sensillar lumen (Figure 11; Steinbrecht 1999). Measurements of the total dendritic circumferences of such branched basiconic ORNs show that they are several times greater than those of unbranched trichoid sensillar ORNs (Figure 10; Rogers et al. 2001).

Capacities of whole-antennal pheromone component–specific channels

Component-specific activity from each type of component-tuned ORN converges onto each type's specifically targeted single glomerulus (Hansson and Anton 2000). The ranges of flux transduction across the suite of ORNs comprising all of the pooled component-specific information about that component from across the antenna will be greater than the range of molecular flux transduction from any individual ORN (cf. Kaissling 1979, 1987; Akers and O'Connell 1988, 1991; Figure 7). This whole-antennal range describes the channel capacity for that component.

Natural turbulence-induced plume-strand flux information has been shown to be preserved in each antennal channel, through the glomeruli and out through projection interneurons (PNs) to higher olfactory centers of the brain (Vickers et al. 2001). The relative abundances of components from each plume strand encounter are assessed by pattern recognition networks in the mushroom body (Szyszka et al. 2005) and lateral protocerebrum (Hildebrand 1996) after receiving fluctuating synchronous reports about the environmental arrival of plume strands that is preserved in the reports of the PNs exiting antennal lobe glomeruli (Vickers et al. 2001). The capability for high-fidelity rendering of the ratios of different odorants occurring as abundance-related flux in plume strands thus depends on the "capacity" of each olfactory channel to carry accurately transduced flux information all the way through to these highest centers. These reports originate with the ORNs' abilities to accurately transduce the relative fluxes of their pheromone components in both high- and low-flux plume strands that are all comprised of the same component ratios originally shed from the point source emitter.

Sex	Sensillum Type (total # / # labeled)		Dendrite Class	n	Mean Dendrite Circumference μm (SD)	Mean Label Density gold/μm (SD)
Male						
	trichodea I (23/23)		A	23	1.7 (0.2)	13.3 (3.3)
			B	27	0.8 (0.2)	3.1 (3.0)
			combined	50	2.7 (0.6)	10.2 (3.0)
	trichodea II (7/7)		A	7	1.2 (0.3)	15.7 (5.6)
			B	9	0.7 (0.1)	7.1 (5.2)
			combined	16	2.0 (0.5)	12.1 (3.9)
	intermediate (4/2)		A'	6	0.9 (0.2)	10.8 (4.8)
			combined	6	2.6 (0.4)	10.5 (5.4)
	basiconic (5/3)		X combined	37	7.5 (3.4)	5.9 (2.5)
Female						
	intermediate (8/8)		A'	22	1.0 (0.2)	9.5 (7.7)
			combined	22	2.7 (0.3)	9.8 (5.0)
	basiconic (9/7)		X combined	49	2.7 (1.2)	0.9 (0.7)

Figure 10 Dendrite sizes (circumferences) of ORNs from *Antheraea polyphemus* male and female sensilla plus mean SNMP gold-labeling densities on the dendrites of ORNs from different types of sensilla (Rogers et al. 2001). Dendrites unlabeled by SNMP antibodies were not considered in these measurements. Male basiconic sensillar dendrites were measured as possessing a significantly larger total circumference from their profuse branching, and therefore were shown to have a larger surface area than the unbranched dendrites from 2 types of trichoid sensilla. "A" refers to the larger spiking ORN in a sensillum and "B" to the smaller spiking ORN.

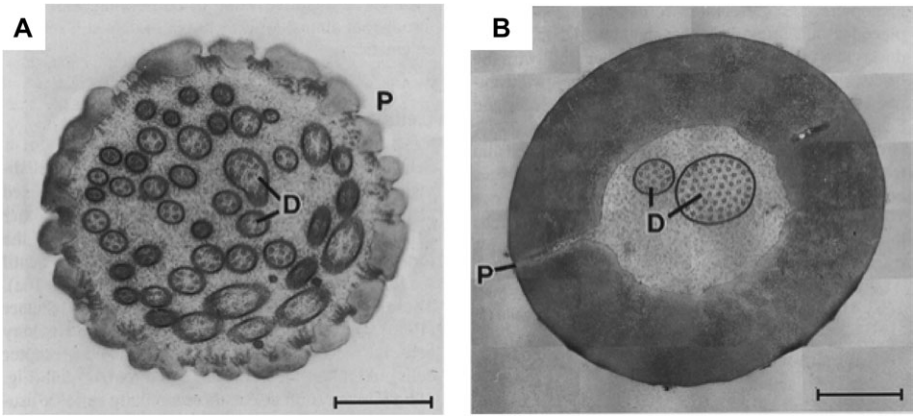

Figure 11 Transmission electron micrographs of: (**A**) a single-wall basiconic sensillum from *Bombyx mori* illustrating the profuse branching of the dendrites of the ORNs housed in this type of sensillum. These dendritic branches fill up most of the space inside the sensillum lumen; (**B**) a trichoid sensillum from a male *B. mori* illustrating the relative sizes of the 2 dendrites housed in it as well as the small amount of space in the sensillum lumen taken up by the dendrites. D = dendrite, P = pore. Figure A is 33 000× and B is 25 000× magnification. From Steinbrecht (1999).

Why more ORNs are needed for the more abundant pheromone component

The overall antennal channel capacity for a component will be reduced if an ORN's working range of component-specific flux transduction can be exceeded. Hence, as we have proposed, major component-tuned ORNs will have been selected to increase the upper end of their working range for their component's oftentimes 10- to 100-fold higher flux. However, as we have seen from in situ recordings of individual ORNs residing in their native sensilla, the sensitivity of a larger spiking ORN is often less than that of its smaller spiking companion ORN (Akers and O'Connell 1991; Cossé et al. 1998; Todd and Baker 1999). There are also cases that deserve further experimentation in which this seems not to be the case (cf. Meng et al. 1989). If the sensitivities of large spiking major component–tuned ORNs are decreased by increasing their upper range of ability to transduce high flux, the whole-antennal channel capacity for that component will have diminished for lower flux. However, for moths that house their ORNs singly in separate sensilla, the olfactory channel capacity for that component can now be restored by increasing their number across the antenna, thereby improving the antennal glomerular reporting ability for low fluxes. These adjustments to optimize whole-antennal channel capacity would explain the greater proportion of major component–tuned ORNs commonly observed across the antennae of noctuid moths compared with the relatively small proportion of minor component–tuned ORNs.

It is noteworthy that even in *O. nubilalis*, in which 2 differently tuned ORNs are cocompartmentalized in most sensilla, the sensilla toward the distal end of the antenna house only a single ORN, and in both the Z strain and the E strain, these single ORNs are tuned to the most abundant component (Hansson et al. 1994). This suggests that even in such species using mostly cocompartmentalized small and large spiking ORNs, component-specific channel capacity can be adjusted, when needed, by singly housing more large spiking major component ORNs to preferentially increase their number relative to minor component–tuned ORNs.

Conclusions and Suggested Future Studies

After first recognizing that evolution will have selected for relatively enormous numbers of pheromone component–tuned ORNs compared with ORNs tuned to general odorants in order to optimize the overall environmental signal-to-noise ratio, for example, "sensitivity," in pheromone communication, we are now proposing that natural selection would have also occurred to maintain fidelity of pheromone olfaction, that is, blend ratio reporting. In light of the widely disparate abundances of the different components that often occur in species' pheromone blends, we are suggesting that the "relative" dendrite sizes and relative abundances of ORN types that are differentially tuned to major and minor sex pheromone components are due to the pheromone olfactory system need to maintain both a wide range of flux transduction capabilities as well as whole-antennal channel capacity of information about each component to produce high-fidelity pheromone "blend ratio reporting" at both low and high pheromone plume-strand flux.

A variety of electrophysiological studies might now be envisioned in order to further test these ideas. These would include performing accurate dose-response measurements on cocompartmentalized large spiking major and small spiking minor pheromone component–tuned ORNs, to compare the working ranges of accurate flux transduction of these ORNs, taking care to measure the emission rates of the different components from the odor cartridges. It should not be assumed that compounds having nearly identical vapor pressures will issue from odor cartridges in

identical amounts, especially if blends in the same cartridge are used. A prediction from such studies would be that large spiking major component–tuned ORNs' absolute sensitivities will be lower than small spiking ORNs, but their upper range will be higher than smaller spiking ORNs. Blend stimuli using ratios similar to those emitted by females should also be used to ensure that natural interactions of odorants on and within sensilla can occur and be recorded in the ORN outputs.

In the process of performing such recordings, other aspects of the sensillar organule for handling flux may be revealed, such as during repetitive stimulation (Barazzo and Kaissling 2002), and carefully monitoring the time courses of action potential spike trains (Kaissling 1986, 1987; Rumbo and Kaissling 1989). The activities of perireceptor factors such as PBPs and PDEs may be able to be deduced from such studies. The absolute sensitivity, flux transduction ranges, and signal-to-noise ratios of ORNs will be dependent in part on the resting action potential frequencies of ORNs. The background firing rates of larger versus smaller diameter dendrites (spike sizes) should be assessed with extra care in such studies.

Concomitant with electrophysiology, more neuroanatomical studies need to be undertaken to measure the relative dendrite sizes (lengths, diameters, surface areas) of noncocompartmentalized major- and minor component–tuned ORNs such as occur in noctuids. The relatively overlooked technique of Kumar and Keil (1996) of microtubular damage in stimulated ORNs should be a good way to explore the relationships between dendrite diameter and spike size and will be especially valuable in physiologically identifying and tagging ORNs housed singly in their own sensilla. This technique will also be valuable for cocompartmentalized ORNs as well because more data on relative dendrite sizes related to spike sizes for many more species need to be accumulated. Because dendrite diameters can vary along their lengths due to constrictions and bleb-like swellings (Keil 1984), serial sections for transmission electron microscopy should be taken along the lengths of the sensilla.

Sensilla housing noncocompartmentalized ORNs that have been physiologically characterized using either the cut-sensillum recording technique (Kaissling 1974; Van der Pers and Den Otter 1978; Lee and Baker 2008) or the tungsten electrode penetration (O'Connell 1975) can be later located and should be examined via scanning electron microscopy to assess pore densities (O'Connell et al. 1983). We would expect that sensilla that house major component–tuned ORNs should have a higher pore density (be more porous) than those housing ORNs tuned to minor components so as to facilitate entry into the sensillum lumen of the molecules in the blend having the highest flux.

As the results of studies accumulate in the coming years on perireceptor factors such as PBPs and PDEs, attention could be paid as much as possible to different forms of these factors occurring in the same sensilla (following Maida et al. 2005). These could be related to differential flux transduction and the influencing of the sensitivity of ORNs that reside in such sensilla. If possible, the relative titers of these forms should be assessed and compared. More data concerning the densities of pheromone-sensitive ORs on dendrites are needed, using SNMPs as possible indicators of such densities (Rogers et al. 2001).

Ideally, more neuroethological studies (cf. Hansson and Baker 1991) should be performed in the field or at least at appropriate downwind distances in a wind tunnel to assess the relationship between initiation and termination of upwind flight responses to the pheromone blend versus single ORN responses to single components in the blend. The studies of Linn et al. (1986, 1987) showed that upwind flight attraction in several species is evoked at lower flux levels (greater distances from the source) of the blend than to individual components or partial pheromone blends. Baker and Roelofs (1981) showed for *Grapholita molesta* how upwind flight terminates at different distanes from the source when the correct blend is emitted at different, but excessive, rates. It would be informative to know for such species how single ORNs tuned to the major and minor pheromone components are responding at these distances of initiation and termination of attraction. Also, Baker et al. (1981) recorded a matrix of *G. molesta* blend ratio and dosage ranges evoking upwind flight that bracketed both the lower threshold levels of upwind flight initiation and the upper thresholds of upwind flight arrestment (termination). Recordings of the differentially tuned ORNs made in the plumes of these behaviorally significant blend ratios and release rates would also be illuminating, and such data for many more species would be valuable for testing the hypothesis of flux transduction capability relative to ORN sensitivity.

We believe there is much to be learned about selection for flux-related sensillar architecture and biochemistry if studies can be focused on better defining the working ranges of flux transduction related to component abundance in pheromone blends. This conceptual platform, encompassing also pheromone component–specific channel capacity across the antenna, may turn out to be translatable to gaining a better understanding of the varied architectures characterizing pheromone-sensitive compared with general odorant-sensitive sensilla. New relationships might come to light involving wall-pore densities, dendritic branchings and surface areas, and overall sensillar morphologies. Perhaps, even the widely varying shapes and architectures of the whole antenna in various moth and insect species might become better understood. These are some of our hopes in proposing this concept.

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