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COMMENTARY

Nearest Neural Neighbors: Moth Sex Pheromone Receptors HR11 and HR13

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Abstract

In moth sex pheromone olfaction systems, there is a stereotypical cocompartmentalization of two or sometimes three olfactory receptor neurons (ORNs) within single trichoid sensilla on which pheromone-sensitive odorant receptors (ORs) are differentially expressed. In this issue of *Chemical Senses*, Krieger et al. show through elegant double and triple in situ hybridization studies that in the moth, *Heliothis virescens*, a pheromone component–related OR (HR11) is expressed on an ORN that is reliably cocompartmentalized in the same sensillum as another OR (HR13) whose ligand is known to be (*Z*)-11-hexadecenal, the *H. virescens* major pheromone component. Although the ligand for HR11 is not yet known, mapping this OR to this particular ORN represents a key advance in piecing together the puzzle of *H. virescens* sex pheromone olfaction.

Introduction

The exquisitely fine odor blend recognition systems typically seen in moth sex pheromone olfaction have been characterized over many decades using behavioral assays and neurophysiological investigations of these very simple 2- or 3-component odor blends. However, functional characterizations of pheromone-related odorant receptors (ORs) to date have been few and far between. Mapping of these ORs to particular olfactory receptor neurons (ORNs) via in situ hybridizations has been instrumental in helping understand sex pheromone olfaction by aligning this information with neurophysiological odor profiles of ORNs and OR functional assays.

In this issue of *Chemical Senses*, Krieger et al. (2009) convincingly demonstrate through double and triple in situ hybridization studies in *Heliothis virescens* males that HR11, a putative sex pheromone-component-tuned OR, is expressed on an ORN that is always colocalized within the same A-type trichoid (hair-like) sensillum as the ORN that expresses HR13, an OR known from prior work to be tuned to the *H. virescens* major sex pheromone component (*Z*)-11-hexadecenal (Z11-16:Ald) (Grosse-Wilde et al. 2007; Kurtovic et al. 2007). Although the ligand for HR11 is still uncharacterized, these new results represent a significant advance in unraveling the complex peripheral sex pheromone olfaction system of *H. virescens* and giving insights into the systems of other heliothine species. Coincidentally, the lack of a known ligand for HR11 is consistent with neurophysiological results that have thus far shown no effective odorants for activating this second, colocalized ORN.

The new study of Krieger et al. (2009) further reports that HR11 and HR13 are colocalized everywhere they appear along the length of the antenna in these A-type sensilla. It also convincingly demonstrates that these two ORs begin to be expressed virtually simultaneously during pupal development up to 2 days earlier than the expression of HR14, HR15, and HR16. The remarkable fidelity of this ORN partner pairing must come to a large degree from as yet poorly understood cascades of transcription factor activity (Ray et al. 2007) that, from the divisions of a single mother cell, orchestrate the construction of each sensillum with its porous cuticle, support cells, and ORNs populated with precisely predesignated ORs (Keil and Steiner 1990).

Advantages of ORN cocompartmentalization to behavior

As discussed by Krieger et al. (2009), the general rule for moth olfactory systems is that ORNs that are paired in the same sensillum type have different rigidly stereotypical tuning profiles, always responsive to the same two different

sex pheromone–related odorants. These two types of molecules may be conspecific pheromone components and involved in attraction of males to that species' pheromone blend. Cocompartmentalization in such cases can serve to optimize sex pheromone blend ratio discrimination, especially when the behavioral tolerance for even slightly off ratios is severely low (Todd and Baker 1999; DeBruyne and Baker 2008). Alternatively, the second cocompartmentalized ORN can be tuned to a heterospecific pheromone component that upon detection by that ORN can act as a behavioral antagonist, reducing upwind flight attraction of males to the sex pheromone blend of their own species. Cocompartmentalizing ORNs of this type is thought to improve accuracy of strand-to-strand reporting of blend quality that can only be optimized by sampling odor strands at the same site at the same time (i.e., in the same sensillum), which minimizes time–space reporting errors (Baker et al. 1998; Todd and Baker 1999).

There is a third occurrence of colocalized secondary ORNs that is not very common. In noctuid moths, there are a few instances in which the ligand to which the ORs on the second cocompartmentalized ORN are tuned is the enzymatic breakdown product of the major sex pheromone component. In both *Agrotis segetum* and *Trichoplusia ni*, the major component is an acetate molecule and the breakdown product is the corresponding alcohol resulting from the activity of a pheromone-degrading esterase present in the sensillum lymph of both species (Prestwich et al. 1989). In both *T. ni* and *A. segetum*, activity by the second ORN results in behavioral antagonism to what otherwise should be a good upwind flight response to the source by males. The colocalized second ORN potentially can report to its glomerulus in the macroglomerular complex (MGC) of the antennal lobe the amounts of breakdown product arising from the flux of major pheromone component across that sensillum.

In moths, all the ORNs known to date that have pheromone-related compounds as ligands arborize in glomeruli located in the MGC and affect male behavior. However, in *H. virescens*, the cocompartmentalized ORN that Krieger et al. (2009) have now shown to express HR11 does not arborize in the MGC (Berg et al. 1998; Lee et al. 2006). Thus, we cannot be sure that the uncharacterized pheromone-related ligand for HR11 is as familiar to us as we might expect. In the closely related species *Heliothis subflexa*, Lee et al. (2006) demonstrated that the second, cocompartmentalized ORN in the *H. subflexa* A-type sensilla never arborizes in the MGC. Rather, these ORNs arborize with 100% fidelity in one particular glomerulus, the "posterior complex 1" (PCx1), that is positioned in the antennal lobe posterior to the MGC within an unusual cluster of glomeruli named the PCx (Figure 1A; Lee et al. 2006). Lee et al. (2006; also Lee 2006) argued that the second colocalized ORN in *H. virescens* A-type sensillum also arborizes in what appears to be a PCx1 glomerulus that is positioned in the same location behind the MGC as in *H. subflexa* (Figure 1B).

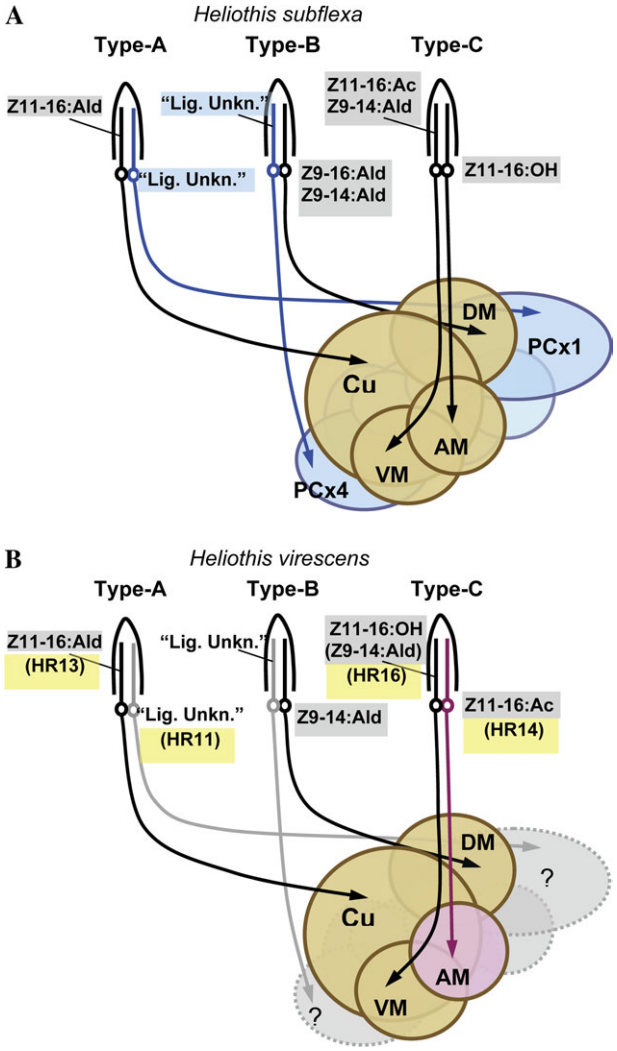

Figure 1 Diagrams of the sensillar compartmentalization arrangements and projection destinations of ORNs in type-A, -B, and -C trichoid sensilla of *Heliothis subflexa* and *Heliothis virescens*. Glomeruli diagramed in gold are MGC glomeruli known to be involved in pheromonal attraction. Cu, cumulus; DM, dorsomedial; VM, ventromedial; and AM, anteromedial (Vickers and Christensen 2003). Glomeruli in pink are MGC glomeruli known to be involved in behavioral antagonism. Glomeruli in blue are those known through anatomical analyses to reside in the PCx and those in gray are speculated to reside in a PCx in *H. virescens* and have not yet been fully anatomically characterized, hence the question marks (?). Pheromone component–related odorants known to optimally activate respective ORNs are noted near each ORN. "Lig. Unkn." next to an ORN signifies that no odorant ligand is known for that ORN. Figure modified from Lee et al. (2006). **(A)** *Heliothis subflexa* showing pheromone component–responsive ORNs (black axons) projecting to their respective MGC glomeruli, and the cocompartmentalized secondary ORNs from types A and B sensilla (blue axons) projecting to the PCx1 and PCx4 glomeruli, respectively. **(B)** *Heliothis virescens* (Berg et al. 1998; Galizia et al. 2000) showing pheromone component–responsive ORNs projecting to respective MGC glomeruli. We speculate from the reports of Berg et al. (1998) that the cocompartmentalized secondary neurons from type-A and type-B sensilla (gray axons) project to PCx1 and PCx4 glomeruli (in gray, called ordinary glomeruli by Berg et al. [1998]; denoted here with question marks). ORs for the diagrammed *H. virescens* ORNs in Type-A and Type-C sensilla are as follows, according to ligand: HR13/Z11-16:Ald; HR11/Lig. Unkn.; HR16/Z11-16:OH(Z9-14:Ald); and HR14/Z11-16:Ac (Krieger et al. 2004, 2009; Grosse-Wilde et al. 2007).

This second ORN in *H. subflexa* was unresponsive to all of the more than 60 odorants to which it was exposed, including different classes of general odorants and all the known heliothine sex pheromone components (Lee 2006; Lee et al. 2006). The second ORN in *H. virescens* expressing HR11 has thus far also proven to be unresponsive to candidate pheromone–related odorants (Berg et al. 1998). Lee et al. (2006; also Lee 2006) guessed that because aldehyde oxidases and dehydrogenases had been identified as degradative enzymes present in heliothine moth sensilla (Prestwich et al. 1989), a likely ligand for the unresponsive cocompartmentalized ORN in *H. subflexa* would be the Z11-16:Ald breakdown product, (*Z*)-11-hexadecenoic acid (Z11-16:COOH). However, initial attempts to stimulate this ORN with various C16 acids, including Z11-16:COOH, resulted in inconclusive results (Lee 2006; Lee et al. 2006). More intensive further testing of this compound using different delivery systems should be undertaken to conclude whether or not it is a ligand for HR11.

Heliothis virescens ORs HR14, HR15, HR16, and HR6

Grosse-Wilde et al. (2007) had functionally characterized the *H. virescens* pheromone–related ORs HR14 and HR16 and along with the results of their double in situ hybridization studies allowed these ORs to be logically assigned to two ORNs that had been neurophysiologically shown to be colocalized in the C-type sensillum (Berg et al. 1998; Baker et al. 2004). Thus, four ORNs expressing their ORs HR11, HR13, HR14, and HR16 are all accounted for in two types of sensilla, A and C.

Out of the group of closely related putative pheromone receptors described by Krieger et al. (2002, 2004), only HR15 and HR6 are now left in limbo. The combined results of Krieger's group (Krieger et al. 2004, 2009; Grosse-Wilde et al. 2007) now place ORNs expressing HR15 as apparently being housed alone in a low proportion of sensilla (Krieger et al. 2004), but the ligand for HR15 is thus far unknown. Coincidentally, the behaviorally important activity of the ORN that responds to the minor pheromone component (*Z*)-9-tetradecenal (Z9-14:Ald) in the third, B type of sensillum (Berg et al. 1995, 1998; Baker et al. 2004) has to date had no functionally characterized ORs assigned to it. In *H. subflexa*, there is a similar ORN in B-type sensilla that responds to the *H. subflexa* minor pheromone component (*Z*)-9-hexadecenal (Z9-16:Ald) and to a lesser degree to Z9-14:Ald (Baker et al. 2004).

Using cobalt backfills, both Berg et al. (1998) in *H. virescens* and Lee et al. (2006) in *H. subflexa* found that there are not one but two ORNs that are cocompartmentalized in B-type sensilla (Figure 1A,B). In *H. virescens*, one ORN responds to Z9-14:Ald (Berg et al. 1995, 1998) and the other was unresponsive to every odorant that was tried (Berg et al. 1998). The Z9-14:Ald-responsive ORN arborizes in the Dorsal Medial (DM) glomerulus of the MGC (Figure 1B), as corroborated by recordings of projection interneurons from the *H. virescens* MGC (Vickers and Christensen 2003). The nonresponsive ORN was shown to consistently arborize in a glomerulus not located in the MGC, which was considered to be an "ordinary" glomerulus (Figure 1B; Berg et al. 1998).

Lee et al. (2006) showed that in *H. subflexa*, the Z9-16:Ald-/Z9-14:Ald-responsive ORN from the B-type sensillum always arborized in the DM glomerulus of its MGC, similar to *H. virescens*. Again, this glomerular assignment was corroborated by recordings of projection interneurons from the *H. subflexa* MGC (Vickers and Christensen 2003). The second, cocompartmentalized ORN in B-type sensilla was unresponsive to every odorant that was tried, just as in *H. virescens*, and it likewise was found to arborize in a glomerulus not located in the MGC (Lee et al. 2006). This glomerulus, named PCx4, (Figure 1A) is located in the PCx and is targeted with 100% fidelity by these unresponsive ORNs. Although Berg et al. (1998) called the arborization destination for the *H. virescens* secondary ORN in B-type sensilla as being an ordinary glomerulus, Lee et al. (2006) noted that this glomerulus seemed to be in the same location as the PCx4 in *H. subflexa* (Figure 1A,B).

In the in situ hybridization studies of Krieger et al. (2004), the low proportion of trichoid sensilla that have ORNs expressing HR15 is not dissimilar to the proportion that neurophysiologically have been characterized as B types that have ORNs responding to Z9-14:Ald (Baker et al. 2004). This suggests that the ligand for HR15 could be Z9-14:Ald. But because there are two ORNs in B-type sensilla, it is possible that this pheromone component might be the ligand for another OR from the HR family, such as HR6. One might expect that the ligand for whichever one of the HRs that is expressed on the as yet unresponsive ORN should be similar to the still unknown ligand for HR11. This conjecture is based on the results from Krieger et al. (2009) that clarify the distribution of *H. virescens* HRs on neuroanatomically characterized ORNs and on the knowledge that the unresponsive cocompartmentalized ORNs from A-type and B-type sensilla project faithfully to non-MGC glomeruli in 2 antennal lobe locations similar to the PCx1 and PCx4 glomeruli in *H. subflexa*.

The impressive work of the Krieger group (Krieger et al. 2002, 2004; Gohl and Krieger 2006; Grosse-Wilde et al. 2007; Krieger et al. 2009) has increasingly provided new pieces helping to fill in the puzzle that is the organization of the *H. virescens* peripheral sex pheromone olfaction system. Krieger et al.'s article in this issue of *Chemical Senses* further clarifies the design of a pheromone detection system of ORs that exhibit a distributed specificity of response to ligands and a stereotypical pattern of expression on cocompartmentalized ORNs within sensilla. The behavioral selection pressures that have resulted in these OR–ORN and ORN–ORN pairings will become clearer in *H. virescens* and other species

by continuing to use an integrative approach to sex pheromone research that includes neuroethological studies coupled with studies of OR function and OR distribution patterns on physiologically characterized ORNs across sensilla.

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