Odor and pheromone detection in *Drosophila melanogaster*

Dean P. Smith

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Abstract *Drosophila melanogaster* has proven to be a useful model system to probe the mechanisms underlying the detection, discrimination, and perception of volatile odorants. The relatively small receptor repertoire of 62 odorant receptors makes the goal of understanding odor responses from the total receptor repertoire approachable in this system, and recent work has been directed toward this goal. In addition, new work not only sheds light but also raises more questions about the initial steps in odor perception in this system. Odorant receptor genes in *Drosophila* are predicted to encode seven transmembrane receptors, but surprising data suggest that these receptors may be inverted in the plasma membrane compared to classical G-protein coupled receptors. Finally, although some *Drosophila* odorant receptors are activated directly by odorant molecules, detection of a volatile pheromone, 11-*cis* vaccenyl acetate requires an extracellular adapter protein called LUSH for activation of pheromone sensitive neurons. Because pheromones are used by insects to trigger mating and other behaviors, these insights may herald new approaches to control behavior in pathogenic and agricultural pest insects.

Keywords Olfaction · Volatile pheromones · Receptors · Signaling · Olfactory

Introduction

Drosophila is proving to be an excellent model system to elucidate essential principles underlying the complex phenomena of odor detection, perception, discrimination, and behavior. Fruit flies have acute olfactory acuity, as evidenced by the presence of escaped laboratory specimens in lab coffee cups and lunches and by the wild animals circling the produce stands in open-air markets. *Drosophila* can identify, orient to, and locate food sources from a distance with a surprisingly limited repertoire of only 62 odorant receptors encoded in the genome [8, 17, 43, 57]. Comparison of vertebrate olfactory anatomy with that of *Drosophila melanogaster* reveals that both types of animals utilize similar strategies to detect and process odor information [23]. Therefore, analysis of the numerically simpler *Drosophila* olfactory system should provide insight into how odors are perceived, how odorant information is discriminated and processed, and ultimately, how odor evokes behavior. This may in turn help us understand our more complex vertebrate olfactory systems. This review will emphasize recent progress in elucidating the early steps of odor and pheromone perception at the molecular and physiological level.

Drosophila and vertebrates use similar strategies to process odor information

The anatomic organization of the *Drosophila* olfactory system shares striking similarities with vertebrate olfactory systems. The organization of the olfactory neurons and the way they are wired to the brain is depicted in Fig. 1. In both types of animals, large families of odorant receptor genes are expressed in primary olfactory neurons. Each olfactory

D. P. Smith (✉)
Department of Pharmacology and Center for Basic Neuroscience,
University of Texas Southwestern Medical Center,
5323 Harry Hines Blvd.,
Dallas, TX 75390-9111, USA
e-mail: Dean.smith@utsouthwestern.edu

Fig. 1 Vertebrates and *Drosophila* share a similar organization in their olfactory systems. **a** Diagram of the *Drosophila* peripheral olfactory system showing olfactory neurons located in sensilla. Note reiterative pairing of specific functional types of neurons within the sensilla. Olfactory neurons expressing the same receptor converge to the same glomerulus in the antennal lobe. Activity in the glomeruli is monitored by projection neurons that innervate higher brain centers in stereotypic patterns. **b** Dendrites of olfactory neurons in vertebrates are exposed to a common fluid into which odorants are dissolved before interacting with the dendrites. Like *Drosophila*, neurons expressing the same receptor converge to the same glomerulus. Glomerular output is relayed to higher olfactory processing centers by mitral cells, which project axons to stereotypic sites in the olfactory cortex (not shown)

neuron chooses a single member of the odor receptor gene family to express. The odorant receptor defines the odor sensitivity of neurons in both *Drosophila* and vertebrates [12, 14, 20, 32, 49, 51, 64]. Each olfactory neuron projects a single axon to the first synaptic relay station in the brain, the antennal lobes in insects, and the olfactory bulb in vertebrates. These relay stations are organized into a large number of glomeruli, which are spherical neuronal structures where primary olfactory neurons synapse with local interneurons and projection neurons that relay glomerular activity to the higher brain centers. Olfactory neurons expressing the same receptor project their axons to the same glomerulus [41, 54, 58]. A glomerulus is usually innervated by one type of receptor-expressing neurons. Therefore, activation of a glomerulus reflects activation of a specific odorant receptor. The current model of odor coding is that an odorant activates specific subsets of receptors, resulting in activation of specific subsets of glomeruli. The brain subsequently “reads” the temporal and spatial activity pattern in the antennal lobe (or olfactory bulb) induced by an odorant, via projection neurons. Indeed, projection neurons (mitral cells in vertebrates) innervate single, specific glomeruli and synapse in stereotypic patterns in the higher odor processing centers [25, 40, 61, 65]. It is thought, therefore, that the brain assigns different glomerular activity patterns unique odor representations or “smells”. The details of how the sensation of “smell” is actually produced by the brain has yet to be determined but may involve production of oscillating activity in olfactory neuronal ensembles [36].

Anatomy of the *Drosophila* peripheral olfactory system

Vertebrate olfactory neuron dendrites are exposed to a common extracellular fluid in the nose. In insects, olfactory neurons are located primarily on the antenna (Fig. 2a).

Fig. 2 Organization of the *Drosophila* peripheral olfactory system. **a** Scanning electron micrograph of a fruit fly. *Ant* Antenna, *palp* maxillary palp. **b** Morphological classes of sensilla located on the antenna. Basiconic (B), coeloconic (C), and trichoid sensilla (T) are

depicted. **c** Drawing of a cross section through a sensillum depicting two olfactory neurons with dendrites projecting into the shaft of the sensillum. The shaft is filled with sensillum lymph secreted from the non-neuronal support cells

Small sets of olfactory neurons are segregated into hair-like structures called sensilla (Fig. 2b). Insects have taken advantage of this segregation to differentially regulate the composition of the extracellular fluid around the olfactory neurons dendrites. In *Drosophila*, the olfactory sensilla are located on the third segment of the antenna and also on the maxillary palps located near the mouth (Fig. 2a) [48]. The olfactory neuron cell bodies are located beneath the cuticle surface of the antenna, but each neuron extends a ciliated dendrite into the shaft of a sensillum (Fig. 2c). The dendrites of between one and four neurons are housed within a single sensillum [48]. The sensilla are filled with a potassium- and protein-rich fluid called sensillum lymph, which bathes the dendrites. The composition of the lymph is regulated by non-neuronal support cells that secrete ions, water, and proteins, including odorant binding proteins into the lymph. The protein composition of this lymph varies in different sensilla (see below).

The antenna contains approximately 1,200 olfactory neurons sequestered in approximately 500 sensilla. The sensilla are divided into three classes based on morphology: the basiconic, the coeloconic, and the trichoid sensilla [48] (Fig. 2b). Functional subtypes within each morphological class are defined by the odorant receptors expressed by the neurons in that sensillum [6, 9–11, 16]. Figure 3 shows a map of the relationship between sensilla types, the receptors expressed in each type, and the glomerulus innervated by neurons expressing those receptors.

To begin to understand how an odorant is detected by an olfactory system in toto, it is essential to understand the odor tuning of individual odorant receptors. This has now been achieved in large part for *Drosophila*. Electrophysiological surveys of antennal sensilla identified functional olfactory neuron types based on observed odor response profiles [6, 11, 20, 63]. However, these surveys do not allow correlation of a specific odor receptor with a functional type of neuron. Odor tuning of a specific *Drosophila* odorant receptor was first estimated by over-expressing receptor Or43a in the antenna [49]. A more accurate approach is to generate mutants defective for expression of specific receptors and determining what functional class was subsequently missing in the antenna. This approach successfully correlated receptor Or43b with the ab8A functional class of olfactory neurons and receptor Or22a with the ab3A functional class [12, 14]. However, generating 60 receptor mutants would be a substantial undertaking. Furthermore, some *Drosophila* olfactory neurons express multiple receptors (see below). Therefore, to more quickly characterize the odor sensitivity of individual odorant receptors, the Carlson group established the “empty neuron” system. Mutants lacking Or22a still have ab3A neurons, but they lack odor responses [20]. By mis-expressing other odorant receptor genes in the ab3A

neurons using the *Or22a* promoter, they effectively “swapped” Or22a with whatever receptor they desired. Then the odor responsiveness of the mis-expressed receptor could be examined using single sensillum electrophysiology and measuring responses to the application of a large odorant panel [11]. The advantage of this approach is that the receptor is assayed in a setting that is very similar to its normal functional environment, which minimizes artifacts of in vitro expression. By matching the response patterns with the mis-expressed individual odor receptors in the empty neuron system with functional neuron types, they were able to define the odor responsiveness for most receptors and correlate these with specific functional olfactory receptor neuron (ORN) classes. These results, together with in-situ hybridization studies mapping *Or* gene expression in the antenna have identified most of the functional types of olfactory neurons in the antenna [9, 16]. Interestingly, the functional types of neurons were found in stereotypical patterns [10, 11]. Neurons expressing particular receptors were always paired with neurons expressing

	sensillum class	receptor	glomerulus
Antenna	Large Basiconic	ab1 92a 42b Gr21a 10a	VA2 DM1 V DL1
		ab2 59b 85a	DM4 DM5
		ab3 22ab 85b	DM2 VM5d
	Small Basiconic	ab4 7a 56a	DL5 DA2
		ab5 82a 47a	VA6 DM3
		ab6 ? 49b	? VA5
		ab7 98a 67c	VM5v VC4
		ab8 43b 9a	VM2 VM3
		ab9 69a 67b	D VA3
		ab10 49a,85f 67a	DL4 DM6
	Coeloconic	ac1 ? ?	? ?
		ac2 ? ?	? ?
		ac3 ? 35a	? VC3
		ac4 ? ?	? ?
Palp	Trichoid	at1 67d	DA1
		at2 23a 83c	DA3 DC3
		at3 2a 19a,b 43a	DA4m DC1 DA4l
		at4 47b 65a,b,c 88a	VA1v DL3 VA1d
	Thin Basiconics	pb1 42a 71a	VM7 VC2
		pb2 33c,85e 46a	VC1 VA7i
		pb3 59c 85d	1 VA4

Fig. 3 Map of functional types of sensilla, the receptors expressed by the neurons in these sensilla, and the glomeruli innervated by these neurons in the antennal lobe. Coeloconic sensilla expressing Or35a have been classified as ac3, according to Yao et al. [63] based on their physiological characterization of coeloconic sensilla (this type is classified as ac1 by Coute et al. [9])

other specific receptors. Therefore, there are fewer different functional types of sensilla than functional types of olfactory neurons [9, 11]. The 22 functional types of basiconic olfactory neurons are located in ten different classes of basiconic sensillum (Fig. 3) [9, 16]. Similarly, the trichoid sensilla fall into four types with nine functional ORN types [9], and coeloconic sensilla come in four types with seven distinct olfactory neuron types [63]. With the exception of Or35a, the receptors expressed by coeloconic sensilla have yet to be correlated with functional neuron types [63].

To understand how odor information is relayed from the olfactory neurons to higher processing centers, we need a wiring diagram that assigns a glomerular target to neurons expressing each odor receptor. The wiring diagram for the *Drosophila* olfactory neurons is nearly complete [9, 16, 58]. Most glomeruli are innervated by olfactory neurons expressing the same particular receptor. However, there are exceptions. Several olfactory neurons express two different receptors (Fig. 3). For example, Or56a and 33a are co-expressed in neurons innervating the DA2 glomerulus [16], Ors 33b and 47a are co-expressed in neurons innervating glomerulus DM3 [16], and Or85e and Or33c are co-expressed in neurons innervating glomerulus VC1 [9, 16]. Two receptors may be co-expressed to broaden the tuning of these neurons. Mis-expression of more than one receptor in a neuron can produce an additive odor response profile [18]. By contrast, Or47a responds to a wider array of odorants when expressed in the empty neurons than in the native neurons where Or33b is co-expressed [20]. In this case, co-expression with Or33b might act to limit the response profile of Or47b. Indeed, expression of Or33b in the empty neuron system reveals that it produces inhibitory responses to most odorants that it detects [20]. Thus co-expression of two receptors in the same neurons can broaden or narrow the odorant response profile of that neuron.

Now that the majority of receptors have been characterized for sensitivity to a large odorant panel, one can ask how does the receptor repertoire as a whole encode odorant quality, quantity and duration. The first attempt to examine the “global” response of the *Drosophila* olfactory system to odors was published recently [21]. In this work, 110 odorants including complex fruit odors, were used at various concentrations to screen 24 receptors expressed in the empty neuron preparation. Whereas 110 odorants are probably a small fraction of the total odor space the system is capable of responding to, this is the first broad probe of the responses of a large fraction of the receptors to odorants. This study found that strong responses to odorants were rare overall, perhaps indicating that individual volatile components do not generally cast overwhelming weight in odor perception. They also revealed that some receptors are broadly tuned, whereas others are narrowly

tuned across the odorant concentrations tested. Surprisingly, inhibitory responses to odorants, where spike frequency decreased upon odorant application, were very common. Remarkably, only three odorants failed to activate or inhibit at least one receptor. Although this may reflect some bias in the selection of odorants (most are present in rotting fruit), it does show that even this small receptor repertoire can cover a significant fraction of odor space. Receptors with similar odor specificity often innervated widely dispersed glomeruli. Most interestingly, these authors attempted to construct a multidimensional odor space model for odor quality, quantity, and duration. They found that positions in this odor space depend most on chemical class of odorant, the concentration, and the molecular complexity of the odorant. Odor concentration correlates with the number of receptors that are activated by an odorant, suggesting that recruitment of additional glomeruli may encode a cue for high odorant concentration. However, as many odorant receptors are inhibitory, perhaps loss of activity in glomeruli connected to inhibitory receptors may also play a role in concentration sensing. It will be extremely interesting to determine if either or both of these mechanisms underlies how these animals chemotax to odorant sources, as disruption of this process, could be of great utility in designing insect repellants.

Signaling mechanisms underlying odorant detection

The signaling mechanisms utilized by primary olfactory neurons in *Drosophila* to transduce the presence of an odorant into action potentials are not known. A small number of mutations have been identified that have dramatic effects on olfactory behaviors. Mutations called *olfD* and *smellblind (sbl)* affect behavioral responses to attractive odorants [1, 38, 39]. Both mutations turn out to be alleles of the sodium channel *para*. Both *sbl* and *olfD* mutations affect a specific splice variant of *para* that appears to primarily affect olfaction. However, it is not clear whether this *para* variant is required in primary olfactory neurons or in higher-level neurons in the odorant pathway. It is likely that the odor insensitivity phenotype in these mutants reflects a loss of action potential transmission in olfactory circuits.

Mutations in a second gene, *retinal degeneration B (rdgB)*, also affect behavioral responses to attractive odorants. *rdgB* encodes a phosphatidylinositol transfer protein involved in inositol phosphate signaling and retinal degeneration in the eye [22, 55]. An allele of *rdgB* was recovered in screens for mutants with olfactory defects [42]. This implicates inositol phosphate signaling in olfaction. However, it is not known if these signaling mechanisms are important for odor detection directly or indirectly.

A third mutation affecting a large fraction of olfactory responses is in *Or83b*. *Or83b* is an odd member of the *Drosophila Or* gene family. *Or83b* is unique in several ways. First, unlike other Ors, it is highly conserved in other insects [26]. Second, it encodes a seven-transmembrane protein with a large loop between predicted transmembrane domains 4 and 5 absent in other members of the *Drosophila Or* family. Third, it is expressed in the majority of olfactory neurons [30, 35]. Finally, *Or83b* alone does not confer odor sensitivity [15]. However, mutations in *Or83b* disrupt trafficking of many other *Or* gene products to the dendrites [35]. Indeed, *Or83b* appears to be a co-receptor for many Ors [35].

These three mutants provide some insight into the possible signal transduction mechanisms underlying *Drosophila* olfaction, but so far, no G proteins or effector enzymes have been identified as primary signal transducers of odor activation of olfactory neurons. What are the biochemical mechanisms underlying odor-induced action potentials?

Drosophila Ors are seven-transmembrane receptors, and it would seem safe to speculate that these receptors, like other receptors in this large gene family, function by activating heterotrimeric G proteins. G_q is an especially attractive candidate, as it is known to mediate phototransduction and inositol signaling in the *Drosophila* eye [37], and splice variants of G_q are expressed in primary olfactory neurons [50]. Indeed, RNA interference knock-down of G_q in primary olfactory neurons resulted in large alterations in the responses of the antenna by electroantennogram recordings [30]. G_o knock-down also results in dramatic defects in odor responses [30]. However, it is not clear if these effects reflect disruption of Or signaling specifically or a more general defect produced by reduced G protein function in olfactory neurons. Whatever the signaling intermediates, it is clear that ion channels are ultimately opened in the olfactory neurons that trigger action potentials.

Recent work from the Vosshall lab raises new questions about the topology of the *Drosophila* odorant receptors in the membrane [3]. The fly receptor gene family is predicted to encode seven transmembrane receptors, but there is virtually no homology with the vertebrate odorant receptor family [7, 17, 57]. Indeed, the *Drosophila Or* family is as similar to vertebrate Ors as ion channels [3].

Benton et al. [3] challenged the assumption that the *Drosophila* Ors are typical seven-transmembrane receptors with the classical structure of the N terminus extracellular and the C terminus on the cytoplasmic side of the membrane. Computer algorithms [52] correctly predicted the classical structure for mouse Ors but predicted that the *Drosophila Or* proteins are reversed in the membrane, with the N termini on the intracellular side and the C terminus on the outside of the cell.

To probe the orientation of the various Or transmembrane segments experimentally, several fusion constructs were expressed with the *LacZ* gene that encodes the enzyme beta-galactosidase. These constructs were expressed in *Drosophila* S2 cells, and the beta-galactosidase reporter substrate X-gal was added. When beta-galactosidase is cytoplasmic, it is enzymatically active, and X-gal is converted into a blue compound. However, the enzyme is inactive when transported across the endoplasmic reticulum lumen or plasma membrane [47]. By fusing *LacZ* to the C terminus of a transmembrane segment and assessing beta-galactosidase activity, the orientation of insertion in the membrane can be determined. The N-terminal portion with the first transmembrane segment of Or9, Or83b, and RH1 (the major *Drosophila* rhodopsin) were fused to *LacZ* and tested. If the N terminus is outside (classical orientation), beta-galactosidase will be cytoplasmic. Indeed, these rhodopsin fusions were blue. However, the Or fusions were not. When a second transmembrane domain was added between the *lacZ* and the first transmembrane domain, the Or constructs were blue, but rhodopsin was not. These results are consistent with the reversed orientation of the first transmembrane region in the membrane compared to classical receptors.

To assess the topology of the full-length proteins, antibodies to specific loops of OR83b were generated, and constructs were expressed in larval salivary glands. Antiserum to specific loops were tested on permeabilized and unpermeabilized cells. Alternatively, GFP was cloned into loops predicted to be inside the cell, and anti-GFP antiserum was applied to unpermeabilized or permeabilized cells. A loop outside the plasma membrane should react to antiserum without detergents (unpermeabilized), whereas intracellular loops should only react with antibodies after detergent lysis of the membrane. The antiserum recognized only the predicted extracellular domains in the unpermeabilized preparations and recognized the intracellular domains only in permeabilized preparations. The results from these studies are consistent with the reversed orientation of the receptors in the membrane.

The only caveat of these experiments is that they do not assess topology of these receptors in the cells that count; the dendrites of the olfactory neurons. To address this, horizontal sections through sensilla were prepared for immunoelectron microscopy and reacted with antipeptide antiserum corresponding to extracellular loop 2 (based on the inverted model). The results showed that this domain is clearly on the outside surface of the dendritic membranes, confirming that this loop is on the outside surface of the dendritic membrane, substantiating the reversed orientation model *in vivo*.

If the receptors are reversed in the membrane, how do they transduce odorant signals? Either there are G-protein

interaction domains present on the opposite loops (compared to classical receptors), allowing ligand-dependent activation of intracellular G proteins or these receptors use unknown mechanisms to trigger action potentials. The answer to this question will require additional studies.

Perception of a volatile pheromone in *Drosophila*: VA detection by T1 sensilla

Insects transmit many diseases including Yellow Fever, West Nile virus, and malaria. Malaria alone is responsible for over a million deaths annually, and most of those are children [59]. In insects and other animals, pheromones trigger specific behavioral responses from other members of the species. In insects, pheromones drive most social behaviors, triggering a plethora of responses ranging from mating, recruitment, and aggregation to aggression and dispersal (reviewed in [53]). The molecular basis for pheromone sensitivity and how pheromones elicit innate behavior is poorly understood, but recent studies on the male pheromone, 11-*cis* vaccenyl acetate (VA), in *Drosophila* provide some novel insights into these mechanisms.

VA mediates aggregation and reproductive behavior in *Drosophila*

The only volatile pheromone identified to date in *Drosophila* is 11-*cis*-VA. This male-specific lipid is present on the cuticle and is secreted by the ejaculatory duct and transferred to females upon mating [4, 5]. VA induces aggregation behavior in both male and female flies [2, 62] and can inhibit mating behavior in males [24]. VA detection is mediated by a small number of olfactory neurons located in specialized trichoid sensilla on the ventral–lateral surface of the *Drosophila* antenna [6, 62].

VA perception: LUSH protein is required for VA detection in vivo

Some, if not most, odorant receptors are activated by odorants directly [20, 49]. By contrast, perception of 11-*cis* VA requires additional factors expressed by non-neuronal support cells, which are secreted into the sensillum lymph. LUSH odorant binding protein is secreted into the sensillum lymph of all trichoid sensilla and is required for VA sensitivity by pheromone sensitive neurons located in T1 sensilla [31, 45, 46, 62]. T1 sensilla contain neurons that are tuned exclusively to VA. In the absence of the LUSH protein, VA fails to elicit aggregation behavior [62]. Even more dramatic is that in the absence of LUSH, VA fails to

elicit action potentials from VA-sensitive neurons [62]. Because the T1 sensilla are tuned exclusively to VA and no other receptors respond significantly to VA [20], this explains the loss of behavioral response to the pheromone. Therefore, in contrast to typical volatile odorants, detection of VA pheromone requires the presence of the extracellular binding protein, LUSH.

LUSH protein triggers activity in Or67d-expressing neurons

What role is LUSH performing in the sensillum lymph that is crucial for VA perception? As LUSH is expressed exclusively in the sensillum lymph of trichoid sensilla (and not by the neurons themselves), logically, LUSH must be functioning in the lymph upstream of the neuronal receptors in the pheromone signal transduction pathway. A number of models are possible for LUSH function. First, LUSH could be a solubilization factor that assists in dissolving hydrophobic VA pheromone molecules into the sensillum lymph. Similar models have been proposed in the moth sex pheromone system, which also utilizes an extracellular binding protein [29, 44, 56, 60]. Similarly, LUSH might serve as a carrier of pheromone molecules in the lymph that delivers the VA cargo to the receptors on the olfactory neuron dendrites [13, 33, 34]. A third alternative is that the LUSH binding protein and VA pheromone form a unique complex that together functions as the ligand for the odorant receptors on pheromone sensitive neurons [27, 28].

A clue to the role of LUSH in pheromone sensitivity came from the surprising finding that LUSH mutants are not only defective for VA sensitivity but the pheromone sensitive neurons also have dramatically reduced spontaneous activity. Indeed, spontaneous activity is reduced over 400-fold in *lush* mutants [62]. Perhaps LUSH is a partial agonist of neuronal receptors and is directly responsible for spontaneous activity. However, other explanations are possible. The reduced spontaneous activity could result from several possible consequences of absence of extracellular LUSH protein. First, perhaps LUSH protein is required for the normal development or identity of these neurons, and the loss of spontaneous activity reflects a developmental abnormality. A second model is that LUSH protein is required for the normal health of the Or67d-expressing neurons, with possibilities ranging from an important osmotic component in the lymph to functioning as a maintenance or growth factor required for neuronal health. Finally, LUSH may be able to directly activate Or67d-expressing neurons.

We know LUSH is secreted into the sensillum lymph in all trichoid sensilla, including the T1, T2A, T2B, and T2C functional types [31, 45, 46]. However, the spontaneous

activity of the T1 neurons is affected in the LUSH mutants, whereas the spontaneous activity in the other types in *lush* mutants is not different from wild-type controls [62]. Therefore, there is a specific consequence of the absence of LUSH on T1 spontaneous activity. If LUSH is a maintenance factor, therefore, it is only required for T1 neurons. These results make an osmotic effect as the source of spontaneous activity loss unlikely, as an identical loss of osmolality is expected in the other subtypes of trichoid sensilla in *lush* mutants.

To further differentiate among the possible models for LUSH on spontaneous neuronal activity in pheromone-sensitive neurons, Xu et al. [62] infused bacterially expressed, recombinant LUSH protein directly into the sensillum lymph of *lush* mutant T1 sensilla using a fine glass recording micropipette. If LUSH is required during development, infusion of recombinant LUSH protein into adult *lush* mutant T1 sensilla should have no effect on spontaneous activity or VA sensitivity. If recombinant LUSH is able to restore function, a developmental role for LUSH on T1 neurons can be definitively eliminated.

When bacterially expressed LUSH protein was infused into *lush* T1 sensilla, spontaneous activity was restored to normal levels within minutes, and VA sensitivity was restored shortly thereafter [62]. Therefore, LUSH does not

function in the development of T1 neurons. Because function is restored in minutes, LUSH is not likely to be a growth or maintenance factor requiring transcription or translation in Or67d-expressing neurons. Therefore, the simplest explanation for these results is that LUSH itself activates Or67d-expressing neurons. Figure 4 shows a working model for LUSH function.

Or67d is the neuronal VA receptor

LUSH is required for VA sensitivity, but LUSH is also present in other trichoid sensilla that are insensitive to VA. Therefore, LUSH, although necessary, is not sufficient for VA detection. What is the neuronal component expressed in T1 neurons that is absent in the other trichoid neurons that mediates VA sensitivity? As odorant receptors determine odor selectivity in *Drosophila* olfactory neurons, a logical possibility is that an odor receptor is specifically expressed in T1 neurons that mediates VA sensitivity. To look for a candidate for the pheromone receptor, Ha and Smith [19] used two developmental mutants that lack T1 sensilla, whereas the other olfactory neuron classes are intact. Using RT-PCR analysis with RNA isolated from antennae from wild type and the two mutants and primers specific to

Fig. 4 Model for LUSH function in T1 sensilla. *Left*, drawing of a T1 sensillum containing a VA sensitive neuron. LUSH proteins (red dots) are secreted by non-neuronal support cells into the sensillum lymph where they are required for pheromone sensitivity. *Right*, LUSH is predicted to exist in two conformations, inactive (red squares) and active (green circles). Only the later form can activate Or67d receptors

(purple circles) located on the dendrites. In the absence of VA pheromone, the inactive form spontaneously flickers into the active form, producing rare single spikes that are observed as spontaneous activity by electrophysiological recordings. VA molecules (yellow spheres) convert or stabilize the LUSH in the activated form, resulting in trains of action potentials

candidate Ors, a single receptor was found to be missing in the two mutants missing T1 sensilla that was present in the wild type. This receptor candidate was Or67d.

These results, combined with RNA in-situ data, demonstrated that Or67d is expressed in T1 neurons. However, this does not prove Or67d is the receptor that mediates VA sensitivity. For example, Or67d might have some unrelated function in T1 neurons. Therefore, to show that Or67d actually mediates VA responses, Ha et al. [19] mis-expressed Or67d in all olfactory neurons. When other trichoid sensilla normally insensitive to VA were tested in these mis-expressing flies, VA induced a dose response that closely mirrored the normal sensitivity of T1 neurons. Importantly, when the mis-expressing flies were crossed into the *lush* mutant background, these responses were lost [19]. These results demonstrate that Or67d is necessary and sufficient to confer VA sensitivity on other olfactory neurons, as long as LUSH is present. Interestingly, when basiconic neurons were tested from flies mis-expressing Or67d in all olfactory neurons, there was no VA sensitivity even when recombinant LUSH protein was infused into the sensillum lymph (Tal Soo Ha and DPS, unpublished observation). This suggests that other trichoid sensillum-specific factors are present, which are also required for VA sensitivity. Screens for mutants that lack VA sensitivity are underway, and at least two novel mutants lacking VA sensitivity have been recovered (Tal Soo Ha and DPS, unpublished observation). It will be interesting to determine if these novel factors together with LUSH and Or67d are sufficient to confer VA sensitivity in basiconic neurons.

The demonstration that a binding protein is required to perceive VA pheromone in *Drosophila* reveals an exciting target for control of disease-carrying or crop-destroying insect pests. Reproductive and social behaviors in insects are usually triggered by pheromones. If pheromones detection requires binding proteins like LUSH in other insects, species-specific agents could be developed to interfere with pheromone signaling. By creating antagonists of these binding proteins that prevent their functioning, the insects will be unable to perceive the pheromone cue. This could be exploited to block mating signals, aggregation on crops, or any number of other pheromone-mediated processes. The appeal of targeting the binding protein is that inhibitors would be unlikely to disrupt the biology of other beneficial insects.

Conclusions and future directions

Our understanding of the organization of the *Drosophila* olfactory system has been advanced greatly over the last few years. Identification and characterization of the *Drosophila* odorant receptor family, with the subsequent

ability to label and map projections of neurons expressing individual receptors, has generated a nearly complete wiring diagram of the peripheral olfactory system. Mis-expression of receptors in the empty neuron system has allowed the odor sensitivity of individual receptors to be evaluated and compared to their responses in their native neurons and to begin evaluation of odor responses across an entire receptor repertoire. Volatile pheromone detection has been shown to require an extracellular adapter protein that may be a direct component of the pheromone signaling pathway. Future work will elucidate if the other 34 members of the odorant binding protein family also mediate pheromone responses in this insect and if similar mechanisms are used in vertebrates. Finally, if the *Drosophila* Ors have reversed membrane topology, how do they signal the presence of odorants to ion channels? With the rate of progress we have witnessed over the last few years, there is no doubt these questions will be answered in the near future.

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