UNIVERSITY OF CALIFORNIA SAN DIEGO

Functional Impact of Sensory Neuron Compartmentalization on Olfactory Behavior

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by

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ABSTRACT of the THESIS

Functional Impact of Sensory Neuron Compartmentalization on Olfactory Behavior

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Master of Science in Biology

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Across animal species, many primary sensory neurons are compartmentalized in specialized structures, such as the olfactory sensory hairs (sensilla) of insects. In *Drosophila,* compartmentalization of olfactory receptor neurons (ORNs) allows grouped neurons to functionally interact through direct electrical interactions such that strong activation of one ORN

can inhibit the olfactory response of its neighbor. However, the functional impact of lateral inhibition on odor-guided behavior remains underexplored. Using thermogenetic behavioral assays, I determine that grouped ORNs in the ac4 sensillum mediate courtship behaviors of opposing valence. In addition, I find that in the oviposition-mediating ab4 sensillum, valence opponency also applies in a courtship context. Furthermore, I conduct pheromone-perfuming experiments and find that detection of antagonistic cues by neighboring ORNs in the at4 sensillum modulates courtship behaviors to a greater degree than detection of antagonistic cues by the non-neighboring at4A and at1 neurons. Taken together, these findings indicate that lateral inhibition in individual sensillum can regulate behavioral responses to countervailing olfactory cues. Thus, this study brings insight into the functional significance of sensory neuron compartmentalization.

Introduction

Environmental stimuli are detected by primary sensory neurons and conveyed as electrical signals to higher brain centers for further processing. The stimulus information is generally believed to be processed entirely in the central nervous system; however, there is evidence to suggest that the sensory periphery may also play a role in signal processing. Across species, many primary sensory neurons in the peripheral nervous system are compartmentalized in specialized structures, such as sensory hairs or taste buds. Interestingly, this compartmentalization is highly prevalent and observed in all major sensory modalities (as reviewed in Ng et al., 2020). Such conservation suggests there is functional importance for this organization beyond simply reflecting developmental constraints. However, the extent to which sensory neuron compartmentalization influences information processing and behavior remains largely unclear.

Compartmentalization is observed across all major sensory systems in *Drosophila melanogaster*, thus making this organism an ideal model for addressing the functional significance for this organization. Specifically, the peripheral olfactory system is especially well-characterized. Fruit flies have two olfactory organs, the antenna and the maxillary palp, which are covered in sensory hairs known as sensilla. Based on morphological and functional features, the olfactory sensilla can be classified into three major subtypes; the basiconic, coeloconic, and trichoid which house olfactory receptor neurons (ORNs) responding to general odorants, acids/amines, and pheromones, respectively (reviewed in Su et al., 2009). Within each sensillum, two to four ORNs, expressing different receptors, are grouped together in a stereotyped manner (de Bruyne et al., 2001). Furthermore, compartmentalized ORNs are designated as A, B, C or D

neurons based on their relative spike amplitudes in descending order as determined through single-sensillum recordings. For example, in the ab2 sensillum which houses two neurons, the large-spike ab2A neuron expresses the Or59b receptor, and the neighboring small-spike ab2B ORN expresses the Or85a receptor (Hallem et al., 2004). The marked stereotypy of ORN pairing at the level of sensillum suggests that such organization is functionally important.

Indeed, compartmentalized ORNs can functionally interact with one another. Specifically, there is intrasensillar, non-synaptic communication in the form of direct electrical interactions that underlie lateral inhibition between neighboring neurons that are co-activated (Su et al., 2012). This lateral inhibition allows neighboring neurons to modify each other's spike activity, and can also modulate behaviors in response to odor mixtures (Su et al., 2012). Such inhibition between neighboring ORNs indicates that at the level of the sensory periphery, grouped ORNs can affect signal processing within a sensillum. Furthermore, lateral inhibition likely also allows individual sensilla to function as initial processing units for olfactory information. Which kind of olfactory information might individual sensilla process? To address this question, it is critical to first determine whether there is an organizing principle within individual sensillum types and, if so, what the purpose of such organization might be.

Interestingly, grouped neurons in the same sensillum likely modulate related behaviors but of opposing valence. Literature survey revealed that large-spike ORN activity generally promotes behavior of positive valence, whereas small-spike neurons typically mediate behavior of negative valence (as reviewed in Ng et al., 2020). However, the behavioral valence of all ORN pairs has not been established. Another graduate student, Shiuan-Tze Wu, is currently addressing whether this organization principle—in which the large-spike neurons signal positive valence, and small-spike neighbors signal negative valence—is conserved for the uncharacterized

neurons. Notably for different sensilla, valence opponency is observed in distinctive behavioral contexts. Identified through previous studies, these contexts are 1) place preference, which determines whether an ORN promotes attractive or aversive behavior (e.g. in the ab1, ab2, ab5, ab9, ac3 sensilla); 2) oviposition (e.g. the ab4 sensillum), and 3) courtship, which tests whether courtship behavior is promoted or inhibited (e.g. in the at4 and ac4 sensilla) (as reviewed in Ng et al., 2020). However, it remains unclear whether grouped ORNs housed in the same sensillum can mediate opposing behaviors in multiple behavioral contexts.

It is also unclear whether valence opponency contributes to olfactory processing in nature where odorants are not presented as single entities, but as complex blends comprising both attractive and aversive cues. This complexity can be observed in pheromone blends from multiple insects. For example, in silkmoths, pheromone blends can be detected by a single sensillum type which houses two ORNs. The large-spike A neuron detects Bombykol, a major attractive sex pheromone component, while its neighboring small-spike B neuron detects Bombkyal, a minor antagonistic sex pheromone component (Kaissling et al., 1978). Importantly, most natural fruit odor blends can simultaneously activate grouped ORNs within the same sensillum (Zhang et al., 2019), suggesting that lateral inhibition in a sensillum plays a general role in processing odor mixtures in addition to pheromone blends. Can co-activation of grouped neurons, mediating opposing valences, result in different behavior responses than when non-grouped neurons, which also mediate antagonistic behaviors, are activated?

This question can be addressed by examining *Drosophila* pheromone detection, which is mediated by specific subsets of sensilla. In the at4 sensillum, grouped ORNs detect different pheromones and mediate courtship behaviors of opposing valence. The at4A ORN, expressing the Or47b receptor, is activated by palmitoleic acid and promotes courtship behavior (van der

Goes van Naters and Carlson, 2007; Lin et al., 2016). The neighboring at4C ORN, expressing the Or88a receptor, is activated by methyl palmitate and inhibits male courtship behavior (Couto et al., 2005; Dweck et al., 2015; van der Goes van Naters and Carlson, 2007; Wu et al., unpublished). On the other hand, the at1 sensillum houses a single ORN, expressing the Or67d receptor, which detects *cis*-vaccenyl acetate (cVA) and inhibits male courtship (van der Goes van Naters and Carlson, 2007; Kurtovic et al., 2007; Tal and Smith, 2006). These well-characterized pheromone-sensing sensilla provide opportunities for testing whether co-activating courtship-related ORNs from the same sensillum can affect behavior to the same degree as activation of non-grouped ORNs from different sensilla.

Another courtship related ORN is housed in the ac4 sensillum. The ac4A neurons, expressing the Ir84a receptor, detect food odors and enhances courtship behavior (Grosjean et al., 2011). However, it is unknown whether the neighboring ac4C neuron mediates behavior of opposing valence. Therefore, it is unclear whether courtship contextual valence opponency is also conserved between grouped ORNs in the ac4 sensillum.

In this project, I address these outstanding questions, specifically: does valence opponency exist in the ac4 sensillum? Is valence opponency conserved in multiple behavioral contexts within a single sensillum type? And lastly, can the co-activation of grouped pheromone sensing ORNs, which mediate opposing valence, affect courtship behavior to a different degree than when ungrouped ORNs from different sensilla are co-activated? By addressing these questions, this study provides key insight into the functional significance of ORN compartmentalization, thereby furthering our understanding regarding olfactory processing in the sensory periphery.

Methods

Drosophila stock:

Flies were raised on standard cornmeal medium containing molasses and kept at 25°C, 50-60% relative humidity in an incubator with a 12-hr light/dark cycle. Experimental flies were collected post eclosion, separated by sex and raised in groups of 10.

Courtship competition assay:

Courtship competition assay was performed as described (Ng et al., 2019) Briefly, three naive males of different genotypes and one virgin Canton-S (CS) female were loaded into a mating chamber (4cm in diameter and 1cm in height, 12.56cm^3 in volume). Mating chambers were placed on top of a Petri dish containing diluted food (50% water). At the base of the mating chamber, mesh gauze separates flies from the food, while providing access to food odors. To differentiate between the male genotypes, two male flies were dusted with fluorescent dyes approximately 24 hours before the experiments. Dye application was alternated between genotypes across experiments to ensure that the fluorescent dust does not impact the behavioral phenotype. Courtship competition assays were conducted at 29-30°C, ~50-60% relative humidity under 660nm red light. For each experiment, 30 groups were set up and flies were allowed 2 hours to copulate. Copulation was visually confirmed, and a UV flashlight was used to determine the fluorescent dye color and thus the mated male's genotype. Data were analyzed using custom MATLAB codes.

Pheromone perfuming experiments

Pheromone perfuming was performed essentially as described (Kurtovic et al., 2007). All pheromone compounds were diluted in acetone. Briefly, 0.3µl of either individual pheromones, or pheromone mixtures, was directly applied to the abdomen of female flies. For negative control groups, acetone was applied instead. Solvent was allowed to evaporate for one hour prior to experiments.

For the single-pair courtship assay, one 7-day-old naive male and one 3-day-old virgin CS female were loaded into a chamber (2cm in diameter and 1cm in height) placed on top of food as described above. Single-pair courtship assay was conducted at 25 °C, ~50% humidity under 660nm red light. Twenty-five pairs were tested per condition per experiment. Copulation was visually confirmed, and the cumulative copulation rate (CCR) was calculated throughout the experimental period. To quantify the pheromone-evoked courtship inhibition. Inhibition index was used to represent the copulation success ratio between the experimental group and control group at the end of the 2-hr assay. The inhibition index was calculated as

$$Inhibition\ index = -\left(\frac{CCR_{experimental} - CCR_{Control}}{CCR_{Control}}\right)$$

Data were analyzed using custom MATLAB codes.

Results

Valence opponency in the ac4 sensillum

First, I investigated whether valence opponency exists in a courtship context in the ac4 sensillum. It is reported that the ac4A ORN can promote male courtship behavior (Grosjean et al., 2011); however, the behavioral phenotypes for the neighboring small-spike ac4B/C neurons has yet to be characterized. To this end, I used a thermogenetic approach in which the warmth-sensing cation channel TrpA1 (Hamada et al., 2008) was expressed in the ac4A or ac4B/C ORNs by using the Ir84a-Gal4 or Ir76a-Gal4 drivers (Benton et al., 2009; Grosjean et al., 2011). Using an established courtship competition assay (Ng et al., 2019), I then pitted a naïve male expressing TrpA1 against two parental control males to determine which fly eventually mated with a virgin wildtype female. Thermogenetic activation of ac4A confirmed that this ORN can promote male courtship behavior (Figure 1A) as reported (Grosjean et al., 2011). In contrast, activation of the neighboring ac4B/C neurons significantly decreased the copulation rate of the transgenic flies when compared to the parental controls (Figure 1B). Together, these results indicate that ac4A and ac4B/C neurons mediate opposing courtship behaviors, further supporting valence opponency as a general organizing principle underlying ORN pairing.

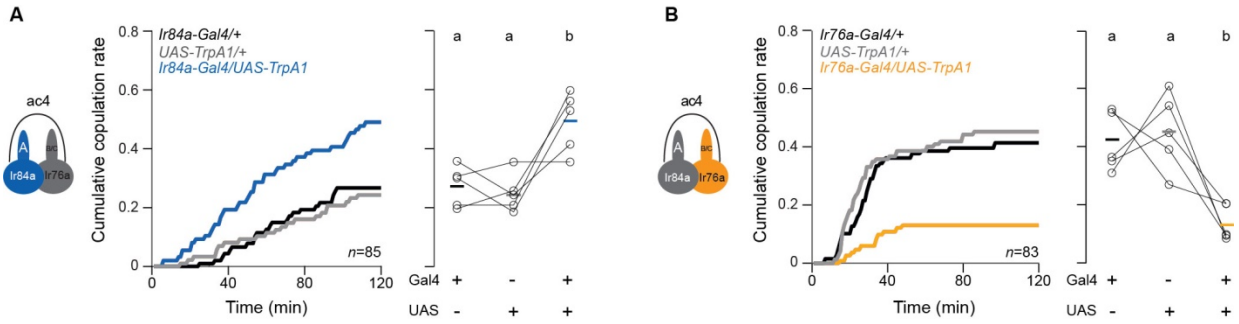

Figure 1. Valence opponency in the ac4 sensillum
Courtship competition assays were conducted with one virgin 3-day Canton-S female and three males of indicated genotypes at ~29°C.
(A) Left: cumulative copulation rates for males with thermogenetically-activated ac4A/Ir84a ORNs compared to parental controls. Right: Copulation rates for the indicated genotypes at the end of the 2-hr assay. Lines connect results from the same experiment (n=5; total 85 matches).
(B) As in (A), except that TrpA1 was expressed in ac4B/Ir76a ORNs (n=5; total 83 matches).
Significant differences ($p < 0.05$) are indicated by different letters; ANOVA followed by Tukey's test.

Individual sensillum types may mediate opposing behaviors in multiple contexts

I then asked whether grouped ORNs in an individual sensillum can mediate opposing behaviors in multiple behavioral contexts. Of note, ORNs housed in the ab1 sensillum can mediate opposing place preference behaviors (Semmelhack and Wang, 2009; Suh et al., 2004, 2007), and it was confirmed that ab1A/Or42b mediates attraction and ab1C/Gr21a mediates aversion using an optogenetic behavioral assay (Wu et al., unpublished). Interestingly, I found that thermogenetic activation of ab1A resulted in no significant courtship modulation in comparison to the parental controls (Figure 2A). Similarly, there was no courtship modulation when the neighboring ab1C ORN was activated (Figure 2B). These results suggest that ORNs housed in the ab1 sensillum do not influence male courtship behavior; thus, valence opponency does not apply to this sensillum in a courtship context.

Next, I examined if valence opponency could be observed in the ab4 sensillum in a courtship context. The ab4A ORN is known to promote female oviposition ((Lin et al., 2015), while the neighboring ab4B ORN inhibits the behavior (Stensmyr et al., 2012). Interestingly,

when thermogenetically activating the ab4A ORN, I observed a significant increase in male courtship behavior (Figure 2C). On the other hand, activation of the neighboring ab4B ORN resulted in significant courtship inhibition (Figure 2D). Therefore, in the ab4 sensillum, valence opponency is observed in two different behavior contexts. Given that courtship and oviposition are both considered as reproductive behaviors, my results may reveal a connection between these two related behaviors.

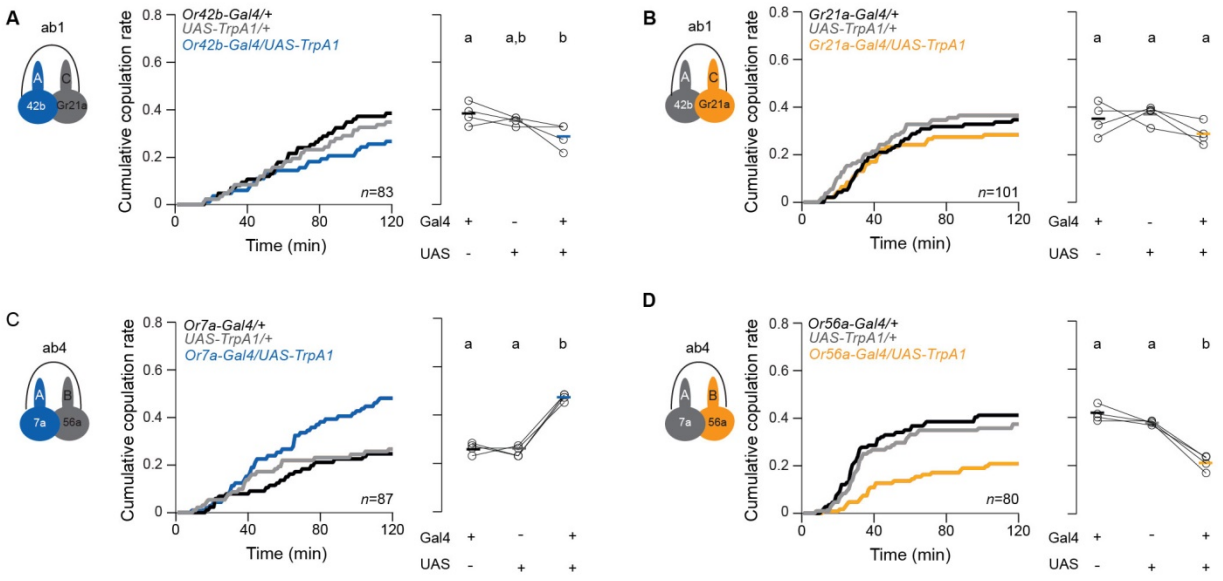

Figure 2. Individual sensillum types may mediate opposing behaviors in multiple contexts
Courtship competition assays were conducted with one virgin 3-day Canton-S female and three males of indicated genotypes at ~29°C.
(A) Left: Cumulative copulation rates for males with thermogenetically-activated ab1A/Or42b ORNs compared to parental controls. Right: Copulation rates for the indicated genotypes at the end of the 2-hr assay. Lines connect results from the same experiment (n=4; total 83 matches).
(B) As in (A), but for males with thermogenetically-activated ab1C/Gr21a ORNs. (n=4; total 101 matches)
(C) As in (A), but for males with thermogenetically-activated ab4A/Or7a ORNs (n=4; total 87 matches)
(D) As in (A), but for males with thermogenetically-activated ab4B/Or56a ORNs (n=4; total 80 matches). Significant differences ($p < 0.05$) are indicated by different letters; ANOVA followed by Tukey's test.

Perfuming females with varying dilutions of aphrodisiac or anti-aphrodisiac pheromones

To investigate how lateral inhibition influences flies' behavioral response to pheromone mixtures, I first sought to determine the dosage effect of different pheromones on male courtship. I focused on three different pheromones: the at4A ligand palmitoleic acid (PA) (Lin et al., 2016), the at4C ligand methyl palmitate (MP) (Dweck et al., 2015), and the at1 ligand *cis*-vaccenyl acetate (cVA) (van der Goes van Naters and Carlson, 2007; Kurtovic et al., 2007). Using a procedure adapted from the Dickson lab (Kurtovic et al., 2007), I directly applied varying dilutions of pheromones or the solvent acetone to female flies before testing them in a single-pair courtship assay with wildtype males.

For the aphrodisiac pheromone palmitoleic acid, significant courtship promotion (~14%) was observed when female flies were perfumed with 0.1 ng of the pheromone ($3x10^{-7}$ dilution) but not at a lower dosage ($3x10^{-8}$ dilution, Figure 3A). Unexpectedly at higher dosages ($3x10^{-6}$ and $3x10^{-5}$), palmitoleic acid appeared to suppress male courtship instead (Figure 3A). The mechanism by which high palmitoleic acid inhibits male courtship remains to be determined (see Discussion). Nevertheless, results from these experiments identified that the $3x10^{-7}$ dilution of palmitoleic acid can be used for the subsequent pheromone mixture experiments.

Next I determined the dosage response relationships for two anti-aphrodisiac pheromones, methyl palmitate and *cis*-vaccenyl acetate, and observed dose-dependent decreases in male courtship behavior: the higher the anti-aphrodisiac pheromones, the more robust the courtship suppression (Figures 3B and 3C). Notably methyl palmitate and *c*VA caused similar degrees of courtship inhibition (13–14%) at $3x10^{-4}$ and $3x10^{-3}$, respectively (Figures 3B and 3C). Therefore, I decided to use these dilutions for the subsequent pheromone mixture experiments.

To verify whether this behavioral modulation was attributed to the select ORNs, I performed the same perfuming experiments with the Or47b, Or88a or Or67d receptor mutant males. The courtship modulation for each of the pheromone dilutions was abolished (lower panels in Figures 3D, E and F). Results from these negative control experiments indicate that the observed courtship modulation indeed arose from activation of the respective ORN types.

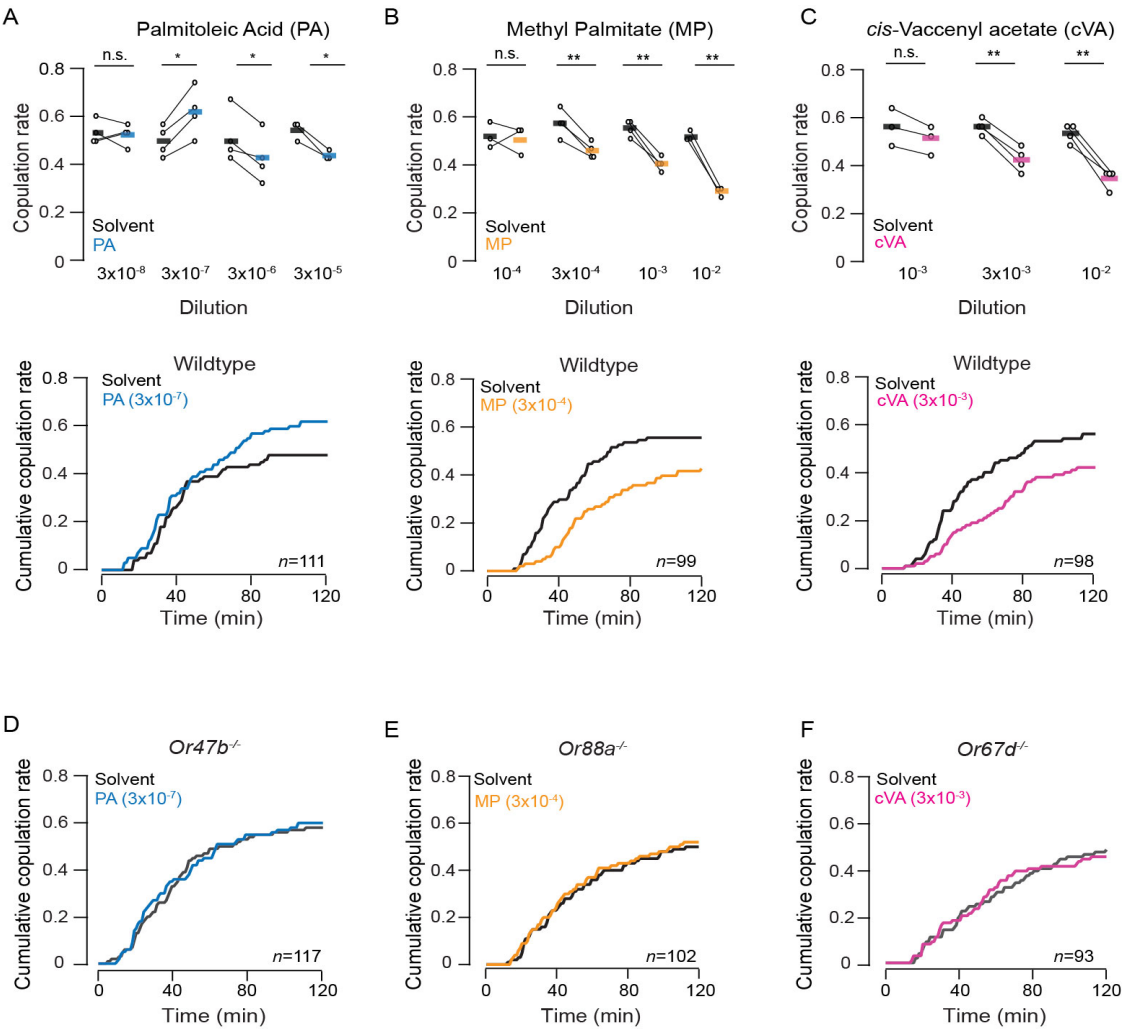

Figure 3. Perfuming females with varying dilutions of aphrodisiac or anti-aphrodisiac pheromones

Single-pair courtship assays were conducted with one 7-day naive Berlin male and one 3-day virgin CS female perfumed with either the solvent or indicated dilutions of pheromone.

(A) Top: copulation rates for males when females were perfumed with the indicated dilutions of palmitoleic acid (PA). Lines connect results from the same experiment. Bottom: cumulative copulation rates when females were perfumed with a $3x10^{-7}$ dilution of PA or solvent control (n=4; total 111 matches).

(B) As in (A), but females were perfumed with the indicated dilutions of methyl palmitate (MP).

(C) As in (A), but females were perfumed with the indicated dilutions of *cis*-vaccenyl acetate (cVA). $^{*}p < 0.05$, $^{**}p < 0.005$, paired t-test.

(D) Cumulative copulation rates for Or47b receptor mutant males when females were perfumed with PA or solvent.

(E) Cumulative copulation rates for Or88a receptor mutant males when females were perfumed with MP or solvent.

(F) Cumulative copulation rates for Or67d receptor mutant males when females were perfumed with cVA or solvent.

Perfuming females with a mixture of aphrodisiac and anti-aphrodisiac pheromones

Given that lateral inhibition in a sensillum provides a means for small-spike, courtship-inhibiting ORN to suppress its large-spike, courtship-promoting neighbor (Su et al., 2012), I hypothesized that co-activation of at4C can suppress at4A-mediated courtship to a greater degree than co-activation of the Or67d ORN housed in a different sensillum (at1). To test this hypothesis, I perfumed female files with a mixture of palmitoleic acid ($3x10^{-7}$) and methyl palmitate ($3x10^{-4}$) or palmitoleic acid and *c*VA ($3x10^{-3}$) and compared the courtship inhibitory effects of these two anti-aphrodisiac pheromones in the presence or absence of palmitoleic acid.

As shown in the previous section, perfuming females with methyl palmitate or *c*VA alone inhibited male courtship (Figures 4A and 4B). To directly compare the degree of courtship inhibition between different pheromones, I determined the inhibition index by normalizing the reduction of copulation rate in each experiment to the copulation rate of the control. As shown in Figure 4C, no significant difference in the inhibition indices was observed between methyl palmitate ($3x10^{-4}$) and cVA ($3x10^{-3}$) when each pheromone was perfumed individually (Figure 4C).

However, when the methyl palmitate or *c*VA was perfumed together with palmitoleic acid, the anti-aphrodisiac effect of these pheromones became markedly different (Figures 4D–F). Specifically, methyl palmitate (at4C ligand) significantly reduced palmitoleic acid (at4A ligand)-elicited courtship by about 30% (Figures 4D and 4F). In contrast, no significant change in male cumulative copulation rate was observed with *c*VA (at1 ligand) when compared to controls (Figure 4E). By comparing the inhibition indices between these two conditions, we found that activating at4C by methyl palmitate can indeed suppress at4A-mediated courtship to a greater

degree than activating at1 ORN by *c*VA (Figure 4F). These results suggest that lateral inhibition can modulate behavioral responses to pheromone mixtures.

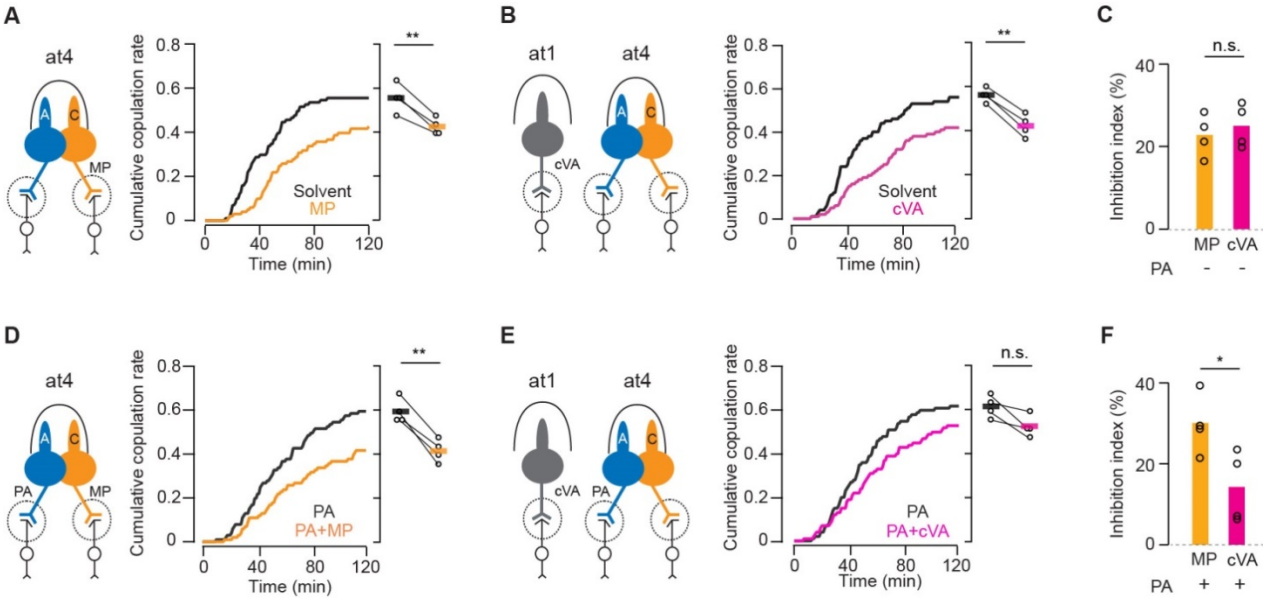

Figure 4. Perfuming females with a mixture of aphrodisiac and anti-aphrodisiac pheromones

Single-pair courtship assays were conducted with one 7-day naïve Berlin male and one 3-day virgin CS female perfumed with either solvent, indicated dilutions of pheromone, or pheromone mixtures. Control single pheromone experiments are shown in (A), (B), and (C); pheromone mixture experiments are shown in (D), (E), and (F)

(A) Left: male cumulative copulation rates when females were perfumed with solvent or methyl palmitate (MP, $3x10^{-4}$ dilution). Right: copulation rates at the end of the 2-hr assay. Lines connect results from the same experiment (n=4; total 99 matches)

(B) As in (A), but females were perfumed with cVA ($3x10^{-3}$ dilution). (n=4; total 98 matches)

(C) Inhibition index from results in (A) and (B).

(D) As in (A), but females were perfumed with a mixture of palmitoleic acid (PA, $3x10^{-7}$ dilution) and MP ($3x10^{-4}$ dilution), or PA alone. (n=4; total 102 matches)

(E) As in (A) but females were perfumed with a mixture of PA ($3x10^{-7}$ dilution) and cVA ($3x10^{-3}$ dilution or PA alone (n=4; total 115 matches).

(F) Inhibition index from results in (D) and (E). $^{*}p < 0.05$, unpaired t-test. $^{*}p < 0.05$, $^{**}p < 0.005$, paired t-test.

Discussion

In this study, I determined that valence opponency exists in the ac4 sensillum in a courtship context. I also found that a single sensillum type can modulate behaviors of opposing valence in multiple behavioral contexts. Lastly, my findings suggest that antagonistic pheromone cues detected by neighboring ORNs can modulate courtship to a greater degree than antagonistic cues detected by non-neighboring ORNs.

Firstly, my results show that valence opponency exists in a courtship context for the ac4 sensillum, which provides further evidence that this organizing principle is conserved across sensillum types (Wu et al., unpublished). Taken together, our combined findings suggest that valence opponency underlies ORN pairing in the *Drosophila* olfactory system. My data thus contributes to the large-scale, systematic behavioral assays which determine the underlying logic of this compartmentalization in the sensory periphery.

In addition, I found that a single sensillum type may mediate opposing behaviors in multiple, related behavioral contexts. Of note, valence opponency has already been observed in an oviposition context for the ab4 sensillum (Lin et al., 2015; Stensmyr et al., 2012). Interestingly, my results show that this organizing principle also applies in a courtship context (Figures 2A and 2B). Given that oviposition and courtship are both considered reproductive behaviors, my findings suggest that this pairing principle in the ab4 sensillum may be broadly important across reproductive behaviors. In contrast, I found that not all sensillum types exhibit valence opponency in multiple contexts. For example, in the ab1 sensillum, this pairing principle was observed in a place preference context (Semmelhack and Wang, 2009; Suh et al., 2004,

2007), but not courtship (Figures 2C and 2D). Thus, these results point to a context specificity to valence opponency and that there are dedicated sensillum types for different behavioral contexts.

Furthermore, I found that different dilutions of anti-aphrodisiac pheromones, methyl palmitate and *c*VA, yielded graded degrees of courtship inhibition (Figure 3). In contrast, a similar monotonic dosage effect was not observed with the aphrodisiac pheromone palmitoleic acid; the pheromone promotes courtship only at a low dosage (0.1 ng) but instead inhibits courtship at high levels (1 or 10 ng, Figure 3A). Given that palmitoleic acid has been demonstrated to be an aphrodisiac pheromone (Lin et al., 2016), why then did I observe both promotion and inhibition of male courtship by this ligand? The simplest interpretation is that activation of large-spike neurons could result in passive activation of unstimulated neighboring neurons, as observed by members in the Su lab (unpublished). Thus, my results raise the interesting possibility that strong activation of Or47b ORNs by high levels of palmitoleic acid can passively activate the neighboring courtship inhibiting Or88a and/or Or65a ORNs, thus resulting in an overall decrease in copulation rate.

Lastly, findings from my pheromone-perfuming experiments indicate that grouped ORNs can regulate behavioral responses to complex odor mixtures. Specifically, I observed that at4A-mediated courtship can be more effectively compromised by co-activating the courtship-inhibiting at4C neuron housed in the same sensillum than co-activating the non-neighboring at1 ORN (Figure 4F). Thus, valence opponency in a sensillum likely functions to enhance contrast between countervailing cues in natural odorants mixtures.

Overall, the results described in this study advance our understanding of sensory neuron compartmentalization in the peripheral olfactory system. In particular, they provide further evidence for the functional significance in the organization of stereotyped ORN pairing. Beyond

Drosophila olfaction, these findings will provide insight into how sensory information is initially processed in the periphery.

This thesis is currently being prepared for submission for publication of the material. Wu, Shiuan-Tze; Martin, Vanessa; Su, Chih-Ying. Figure four was co-authored with Wu, Shiuan-Tze.

References

Benton, R., Vannice, K.S., Gomez-Diaz, C., and Vosshall, L.B. (2009). Variant Ionotropic Glutamate Receptors as Chemosensory Receptors in Drosophila. Cell *136*, 149–162.

De Bruyne, M., Foster, K., and Carlson, J.R. (2001). Odor coding in the Drosophila antenna. Neuron *30*, 537–552.

Couto, A., Alenius, M., and Dickson, B.J. (2005). Molecular, anatomical, and functional organization of the Drosophila olfactory system. Curr. Biol. *15*, 1535–1547.

Dweck, H.K.M., Ebrahim, S.A.M., Thoma, M., Mohamed, A.A.M., Keesey, I.W., Trona, F., Lavista-Llanos, S., Svatoš, A., Sachse, S., Knaden, M., Hansson, B. (2015). Pheromones mediating copulation and attraction in Drosophila. Proc. Natl. Acad. Sci. U. S. A. *112*, E2829–E2835.

van der Goes van Naters, W., and Carlson, J.R.R. (2007). Receptors and Neurons for Fly Odors in Drosophila. Curr. Biol. *17*, 606–612.

Grosjean, Y., Rytz, R., Farine, J.P., Abuin, L., Cortot, J., Jefferis, G.S.X.E., and Benton, R. (2011). An olfactory receptor for food-derived odours promotes male courtship in Drosophila. Nature *478*, 236–240.

Hallem, E.A., Ho, M.G., and Carlson, J.R. (2004). The molecular basis of odor coding in the Drosophila antenna. Cell *117*, 965–979.

Hamada, F.N., Rosenzweig, M., Kang, K., Pulver, S.R., Ghezzi, A., Jegla, T.J., and Garrity, P.A. (2008). An internal thermal sensor controlling temperature preference in Drosophila. Nature *454*, 217–220.

Kaissling, K.E., Kasang, G., Bestmann, H.J., Stransky, W., and Vostrowsky, O. (1978). A new pheromone of the silkworm moth Bombyx mori - Sensory pathway and behavioral effect. Naturwissenschaften *65*, 382–384.

Kurtovic, A., Widmer, A., and Dickson, B.J. (2007). A single class of olfactory neurons mediates behavioural responses to a Drosophila sex pheromone. Nature *446*, 542–546.

Lin, C.-C., Prokop-Prigge, K.A., Preti, G., and Potter, C.J. (2015). Food odors trigger Drosophila males to deposit a pheromone that guides aggregation and female oviposition decisions. Elife *4*, 1–26.

Lin, H.H., Cao, D.S., Sethi, S., Zeng, Z., Chin, J.S.R., Chakraborty, T.S., Shepherd, A.K., Nguyen, C.A., Yew, J.Y., Su, C.Y., Wang, J. (2016). Hormonal Modulation of

Pheromone Detection Enhances Male Courtship Success. Neuron *90*, 1272–1285.

Ng, R., Salem, S.S., Wu, S.T., Wu, M., Lin, H.H., Shepherd, A.K., Joiner, W.J., Wang, J.W., and Su, C.Y. (2019). Amplification of Drosophila Olfactory Responses by a DEG/ENaC Channel. Neuron *104*, 947-959.e5.

Ng, R., Wu, S.T., and Su, C.Y. (2020). Neuronal Compartmentalization: A Means to Integrate Sensory Input at the Earliest Stage of Information Processing? BioEssays *2000026*, 1–12.

Semmelhack, J.L., and Wang, J.W. (2009). Select Drosophila glomeruli mediate innate olfactory attraction and aversion. Nature *459*, 218–223.

Stensmyr, M.C., Dweck, H.K.M., Farhan, A., Ibba, I., Strutz, A., Mukunda, L., Linz, J., Grabe, V., Steck, K., Lavista-Llanos, S., Wicher, D., Sachse, S., Knaden, M., Becher, P., Seki, Y., Hansson, B. (2012). A conserved dedicated olfactory circuit for detecting harmful microbes in drosophila. Cell *151*, 1345–1357.

Su, C.Y., Menuz, K., and Carlson, J.R. (2009). Olfactory Perception: Receptors, Cells, and Circuits. Cell *139*, 45–59.

Su, C.Y., Menuz, K., Reisert, J., and Carlson, J.R. (2012). Non-synaptic inhibition between grouped neurons in an olfactory circuit. Nature *492*, 66–71.

Suh, G.S.B., Wong, A.M., Hergarden, A.C., Wang, J.W., Simon, A.F., Benzer, S., Axel, R., and Anderson, D.J. (2004). A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila. Nature *431*, 854–859.

Suh, G.S.B., Ben-Tabou de Leon, S., Tanimoto, H., Fiala, A., Benzer, S., and Anderson, D.J. (2007). Light Activation of an Innate Olfactory Avoidance Response in Drosophila. Curr. Biol. *17*, 905–908.

Tal, S.H., and Smith, D.P. (2006). A pheromone receptor mediates 11-cis-vaccenyl acetate-induced responses in Drosophila. J. Neurosci. *26*, 8727–8733.

Zhang, Y., Tsang, T.K., Bushong, E.A., Chu, L.A., Chiang, A.S., Ellisman, M.H., Reingruber, J., and Su, C.Y. (2019). Asymmetric ephaptic inhibition between compartmentalized olfactory receptor neurons. Nat. Commun. *10*, 1–16.