Robust olfactory responses in the absence of odorant binding proteins

Shuke Xiao, Jennifer S. Sun, and John R. Carlson

Department of Molecular, Cellular, and Developmental Biology, Yale University

Abstract

Odorant binding proteins (Obps) are expressed at extremely high levels in the antennae of insects, and have long been believed essential for carrying hydrophobic odorants to odor receptors through the aqueous sensillar lymph. Previously we constructed a map of the *Drosophila* antenna, in which the abundant Obps are mapped to olfactory sensilla with defined functions. Here we have deleted all the *Obp* genes that are abundantly expressed in the antennal basiconic sensilla, which respond to fruit odors. We then tested six functional types of sensilla systematically in the mutants and found that all respond robustly. Odors that are diverse in chemical and temporal structures all elicited strong responses. One sensillum that responds to an odorant that affects oviposition gives a greater physiological response following deletion of *Obp* genes. Moreover, this mutant shows a greater oviposition response to the odorant. Our results support a model in which many sensilla can respond to odorants in the absence of Obps, and many Obps are not essential for olfactory response, but that some Obps can modulate olfactory physiology and the behavior that it drives.

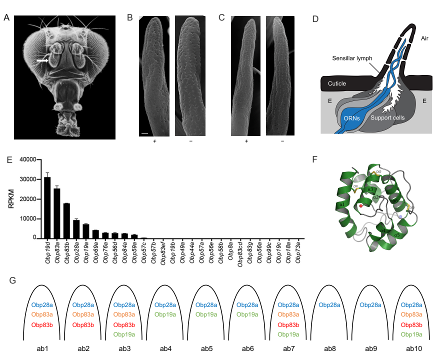

Figure 1. Organization of the *Drosophila* antenna and morphology of mutant olfactory sensilla.
(A) Scanning electron micrograph of a fly head, arrow indicates antenna. Adapted from https://www.sdbonline.org/sites/fly/aimain/wild-type-head.jpg. **(B)** Large basiconic sensilla of control (left) and of an *Obp19a⁻; Obp28a⁻; Obp83a⁻,b⁻* mutant (right). Scale bar is 0.5 µm and also applies to (C). **(C)** Thin basiconic sensilla of control (left) and an *Obp19a⁻; Obp28a⁻; Obp83a⁻,b⁻* mutant (right). **(D)** Olfactory sensillum. Adapted from (Larter et al., 2016) and (Shanbhag et al., 1999). E=Epidermal cells. **(E)** Members of the *Obp* gene family detected in the third antennal segment, where olfactory sensilla are located, at >1 read per million mapped reads in each of three samples. Genes are listed in decreasing order of expression level, indicated in terms of reads per million mapped reads per kilobase of gene length (RPKM). Adapted from (Menuz et al., 2014). **(F)** Structure of an Obp. Obp1 of Aedes aegypti is visualized with six α-helices (α1–6) and three disulfide linkages (DS1–3) labelled. Adapted from (Leite et al., 2009). **(G)** Obp-to-sensillum map of the 10 functional types of basiconic sensilla.

Specific aims

Aim 1. Generate mutant basiconic sensilla without abundant *Obps*

Aim 2. Determine physiological effects of mutating abundant *Obp* genes

Aim 3. Characterize the molecular mechanism of *Obp* action.

Results

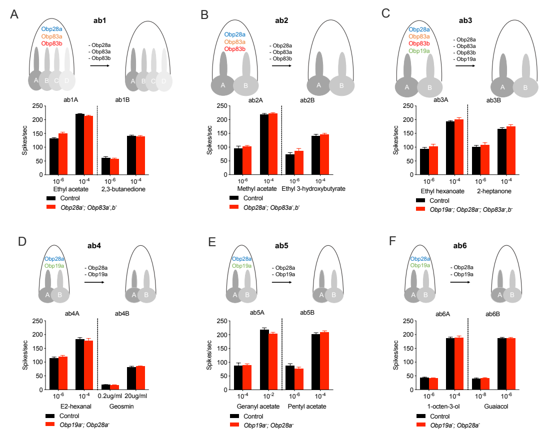

Figure 2. Responses of individual ORNs in each type of sensillum after removal of abundant Obps.
(A-F) ab1-ab6. Responses (mean ± SEM, spikes/s) are to 0.5s pulses of strong ligands (n = 6). No significant differences were found by the Mann-Whitney U test.

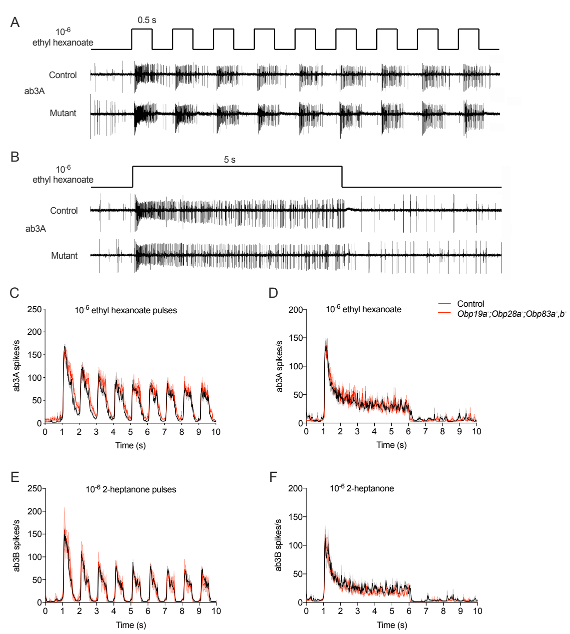

Figure 3. Responses to stimuli of different temporal dynamics in mutant ab3 sensilla
(A) Consecutive 500ms pulses and representative responses to ethyl hexanoate, which activates ab3A, in control and *Obp19a⁻; Obp28a⁻; Obp83a⁻,b⁻* flies. **(B)** Responses to a 5s pulse. **(C)** Peri-stimulus time histogram (PSTH) of ab3A responses to consecutive 500ms pulses of 10^{-6} ethyl hexanoate delivered at 1 Hz. **(D)** A PSTH of ab3B responses to a 5s pulse of 10^{-6} 2-heptanone, n = 4. **(E)** PSTH of ab3B responses to consecutive 500ms pulses of 10^{-6} 2-heptanone, n = 5. **(F)** PSTH of ab3A responses to a 5s 10^{-6} ethyl hexanoate pulse, n = 5. The bin sizes for PSTHs is 25ms. Shaded areas surrounding each curve indicate SEM.

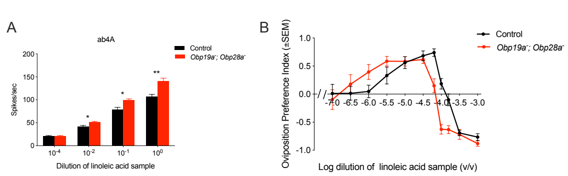

Figure 4. *Obp19a⁻; Obp28a⁻* flies have stronger responses to a sample of linoleic acid.
(A) Physiological responses of ab4 sensilla to a sample of linoleic acid are stronger across several concentrations than control responses. * $p < 0.05$, ** $p < 0.01$, non-parametric t test, n = 5. **(B)** Shift in oviposition preference for a linoleic acid sample in *Obp19a⁻; Obp28a⁻* mutants.

Summary of Results

Six types of sensilla respond robustly in the absence of Obps to many odor stimuli with different chemical and temporal structure

One odor which elicits a stronger response also triggers a stronger behavioral output

These results challenge the long-held paradigm that odors must be delivered to odor receptors by Obps

Some Obps can modulate olfactory physiology and the behavior that it drives.

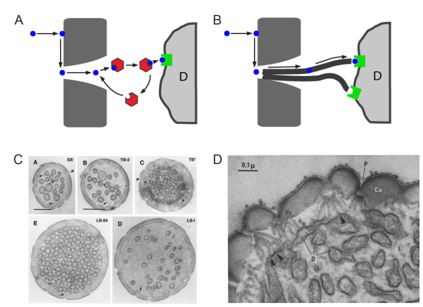

Figure 5. Models of odorant transport via Obps or pore tubules
(A) Odorant transport via Obps in a sensillum. An odorant molecule contacts the membrane, diffuses in the surface of the cuticle (dark gray) until it reaches a pore, enters the sensillum lymph through the pore, binds to an Obp (red hexagon), and is transported to an olfactory receptor (green square) on an ORN dendrite (D). **(B)** Odorant transport via pore tubules. An odorant molecule contacts and diffuses on the cuticle surface, enters a pore, and is transported along a pore tubule to an olfactory receptor on a dendrite (D). An Obp could bind to the odorant at any point after the odorant reaches the pore. **(C)** Dendritic branching of ORNs in different basiconic sensilla. Adapted from (Shanbhag et al., 1999). **(D)** Transmission electron micrograph of the pore (P) and pore tubules (Pt) of an olfactory sensillum of *Bombyx mori.* Adapted from (Steinbrecht 1973).

Ongoing Investigations

Molecular mechanism of Obp function

- Immunoprecipitation of Obps and their potential protein binding partners
- Identify the binding affinity of Obps with ligands
- Resolve structures with/without bound ligands

References

Larter, N.K., Sun, J.S., and Carlson, J.R. (2016). Organization and function of Drosophila odorant binding proteins. *eLife 5*: e20242.

Leite, N.R., et al. (2009). Structure of an odorant-binding protein from the mosquito Aedes aegypti suggests a binding pocket covered by a pH-sensitive "Lid". *PLoS One 26*; 4(11): e8006.

Shanbhag, S.R., Müller, B., and Steinbrecht, R.A. (1999). Atlas of olfactory organs of Drosophila melanogaster 1. Types, external organization, innervation and distribution of olfactory sensilla. *International Journal of Insect Morphology and Embryology 28*, 377-397.

Steinbrecht, R.A. (1973). Der feinbau olfaktorischer sensillen des Seidenspinners (Insecta, Lepidoptera). *Zeitschrift für Zellforschung und Mikroskopische Anatomie 4*, 533-565.

Sun, J.S.*, Xiao* S, and Carlson, J.R. (2018). The diverse small proteins called odorant-binding proteins. *Open Biology 8(12):* 180208.

Acknowledgements

We thank Zina Berman for technical support. We thank Dr. Melissa Harrison, Dr. Kate O'Connor-Giles, Dr. Jill Wildonger, and Dr. Simon Bullock for plasmids pDsRed-attP (Addgene plasmid 51019) and pCFD4-U6:1_U6:3tandemgRNAs (Addgene plasmid 49411). We thank Drs. Hany Dweck, Mahmut Demir, Neeraj Soni, and Srinivas Gorur-Shandilya for help with electrophysiology.

This work was supported by the China Scholarship Council-Yale World Scholars Program (X.S.), by an NSF Graduate Research Fellowship (J.S.S.), by the Dwight N. and Noyes D. Clark Scholarship Fund (J.S.S.), by a Scholar Award from the International Chapter of the P.E.O. Sisterhood (J.S.S.), and by NIH DC02174, NIH DC04729, and NIH DC11697 (J.R.C.)