Title
Olfactory neuromodulation of motion vision circuitry in Drosophila.

Permalink
https://escholarship.org/uc/item/4xb2w4ct

Journal
Current biology : CB, 25(4)

ISSN
0960-9822

Authors
Wasserman, Sara M
Aptekar, Jacob W
Lu, Patrick
et al.

Publication Date
2015-02-01

DOI
10.1016/j.cub.2014.12.012

Peer reviewed
Olfactory Neuromodulation of Motion Vision Circuitry in *Drosophila*

**Highlights**
- Small-field motion detection neurons are required for odor-tracking behavior
- Responses of a directional wide-field interneuron (Hx) increase with paired odor
- Odor activates octopaminergic (OA) neurons that innervate the visual system
- OA cells contact Hx; OA vesicle trafficking is required for odor-tracking behavior

**Authors**
Sara M. Wasserman, Jacob W. Aptekar, ..., Camilla Larsen, Mark A. Frye

**Correspondence**
frye@ucla.edu

**In Brief**
Wasserman et al. report that a directionally selective wide-field motion-detecting neuron (Hx) in the fly increases response gain with paired odor. This multimodal interaction is dependent upon vesicle trafficking from octopaminergic neurons, which are themselves responsive to odor and make cell-cell contact with Hx.
Report

Olfactory Neuromodulation of Motion Vision Circuitry in Drosophila

Sara M. Wasserman,1,4 Jacob W. Aptekar,1,4 Patrick Lu,1 Jade Nguyen,1 Austin L. Wang,1 Mehmet F. Keles,3 Anna Grygoruk,2 David E. Krantz,2 Camilla Larsen,3 and Mark A. Frye1,4

1Howard Hughes Medical Institute and Department of Integrative Biology and Physiology, University of California, Los Angeles, Los Angeles, CA 90095, USA
2Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA
3Medical Research Council Centre for Developmental Biology, King’s College London, London SE1 1UL, UK

Summary

It is well established that perception is largely multisensory [1]; often served by modalities such as touch, vision, and hearing that detect stimuli emanating from a common point in space [2, 3]; and processed by brain tissue maps that are spatially aligned [4]. However, the neural interactions among modalities that share no spatial stimulus domain yet are essential for robust perception within noisy environments remain uncharacterized. Drosophila melanogaster makes its living navigating food odor plumes. Odor acts to increase the strength of gaze-stabilizing optomotor reflexes [5] to keep the animal aligned within an invisible plume, facilitating odor localization in free flight [6–8]. Here, we investigate the cellular mechanism for cross-modal behavioral interactions. We characterize a wide-field motion-selective interneuron of the lobula plate that shares anatomical and physiological similarities with the “Hx” neuron identified in larger flies [9, 10]. Drosophila Hx exhibits cross-modal enhancement of visual responses by paired odor, and presynaptic inputs to the lobula plate are required for behavioral odor tracking but are not themselves the target of odor modulation, nor is the neighboring wide-field “HSE” neuron [11]. Octopaminergic neurons mediating increased visual responses upon flight initiation [12] also show odor-evoked calcium modulations and form connections with Hx dendrites. Finally, restoring synaptic vesicle trafficking within the octopaminergic neurons of animals carrying a null mutation for all amineergic signaling [13] is sufficient to restore odor-tracking behavior. These results are the first to demonstrate cellular mechanisms underlying visual-olfactory integration required for odor localization in fruit flies, which may be representative of adaptive multisensory interactions across taxa.

Results and Discussion

In addition to feedback from head movements [14–18], a fly in flight stabilizes its gaze by optomotor steering movements of the wings that turn the whole body [19]. The strength of steering optomotor responses increases when flies experience an appetitive odor [5]. Here we tethered a fly rigidly within a flight simulator composed of a wrap-around electronic display and equipped with an odor port (Figure 1A) to measure the optomotor impulse response to a rapid rotation of the visual panorama [21]. Pairing an appetitive food odor (vapor) with the visual stimulus results in a roughly 40% increase in the optomotor response (OMR), which is assessed by measuring the mean difference in wing beat amplitude across the two wings (ΔWBA) elicited by an impulse in yaw velocity (Figure 1B), consistent with prior measurements [5].

Optomotor responses in Drosophila can be elicited by optogenetic activation of tangential wide-field collating neurons HSE and HSN housed in the third optic ganglion, the lobula plate [22]. To examine whether motion integrating circuitry of the lobula plate is involved in odor-enhanced OMRs, we genetically hyperpolarized the small-field columnar neurons T4 and T5, which supply retinotopic motion signals to the lobula plate [23]. Using the same magnetic–tether flight simulator (Figure 1C) applied to demonstrate the dependence of self-generated visual motion signals for active plume tracking [24], we measured the animals’ ability to locate and stabilize their heading within a vinegar plume. We divided plume-tracking behavior into three components: (1) initial detection, defined by the proportion of flies that oriented themselves within ±10° of the odor nozzle—flies that did not do so were not included in the subsequent analysis; (2) acquisition, defined by time spent within the plume over the first 10 s of the trial; and (3) continuous tracking, defined by how much of the final 10 s of the trial the fly spent oriented within the plume (Figure 1D). We found no significant difference between the proportions of T4T5-blocked versus control flies that detected the plume (chi-square test, p > 0.05). Similarly, blocking T4T5 did not significantly alter the mean time spent in the plume during the acquisition phase, but T4T5-blocked flies were unable to sustain plume tracking for the duration of the trial, in comparison to controls (Figures 1D and 1E). This shows that whereas the lack of motion signals carried by T4T5 to the lobula plate does not compromise the animals’ ability to detect or initially localize an odor plume, local motion signals are required to stabilize flight heading within the plume. This is consistent with the finding that switching the high-contrast grating displayed in the flight arena to an equiluminant grayscale, thereby reducing optic flow generated by the fly’s own movements, eliminates its ability to remain within the plume [24].

A lobula plate tangential cell (LPTC) was recently identified anatomically in Drosophila, along with a number of neurons within higher-order olfactory regions of the mushroom bodies, by its shared expression of the Odd-skipped transcription factor [9]. The tangential dendritic arbor of this LPTC spans the dorsal projection of the lobula plate (Figure 2Bi), tightly restricted to layer 2 (Figures 2Bii and 2Biii), which is the layer receiving back-to-front directional motion input from the columnar T4T5 terminals [23]. The axon projects heterolateral to the cell body and dendrites [9]. To characterize its motion-coding properties, we expressed a genetically encoded calcium indicator, GCaMP6m [25], under the Odd-Gal4 driver [9] and recorded cellular activity under a two-photon excitation

*Correspondence: frye@ucla.edu
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
Imaging from dendritic regions of interest (ROIs) (see Experimental Procedures) in response to a narrow vertical bar, we demonstrate that this cell is excited by back-to-front motion across the ipsilateral eye within a 50° receptive field positioned just ipsilateral to the visual midline (Figure 2C) and is more excited by progressively wider randomly textured bars (Figure 2D). We found no systematic response differences within small ROIs spanning the tangential dendritic arbor (data not shown) and therefore focused subsequent imaging analysis on a primary dendritic branch that was identifiable in each preparation (Figure 2B, white box). To further explore wide-field response properties, we varied the orientation of a full-field grating, demonstrating that this cell is strongly tuned to front-to-back motion oriented along the horizontal body axis (Figure 2E) and, like other wide-field Drosophila LPTCs [11, 23], exhibits a 1 Hz temporal frequency optimum (Figure 2F).

Motivated by the transcription-factor spatial profile shared with higher-order olfactory projection neurons, we sought to determine whether Hx was cross-modally activated by odor. The two-photon recording preparation and LED display was equipped with a laminar flow olfactometer (Figure 2A). We presented a regime of five repeated 10-s epochs of back-to-front wide-field motion interspersed with rest periods. The second motion epoch was accompanied by a 10-s odor pulse (delivered bilaterally). There was a subtle yet significant increase in the motion-elicited excitatory response of Hx during paired odor presentation (epoch 2, Figure 3A), observed within each individual fly preparation tested (Figure 3B) but absent in water vapor controls (Figure 3C). To determine whether the primary site of visual-olfactory integration resided with Hx or the local motion detectors presynaptic to the lobula plate, we performed the same experiment and recorded the intracellular activity of T4T5 cells. The T4T5-Gal4 driver labels cell processes within the medulla, lobula, and lobula plate ([22, 23] and Figure 3D), and we found no differences between responses from ROIs imaged within the processes of these neuropils (chi-square test, p > 0.05), nor did we observe any changes in the excitatory motion responses of T4T5 ROIs found within the lobula plate upon paired odor presentation (Figures 3E and 3F). These results reject the possibility that odor-enhanced responses in Hx represent general arousal phenomena and confirm that the site of cross-modal interaction resides within wide-field-integrating lobula plate neurons rather than presynaptic local motion detectors. To assess whether odor activates all LPTCs, we examined the activity of HSE, a neighboring wide-field-integrating lobula plate neuron rather than presynaptic local motion detectors. To assess whether odor activates all LPTCs, we examined the activity of HSE, a neighboring neuron to Hx that is selective for horizontal motion (HSE [11]; Figure 3G), but we did not observe odor-evoked changes in the visual responses of this cell (Figures 3H and 3I).

Visual responses by LPTCs are modulated by the onset of locomotion [27, 28], and this increase in response gain is mediated by octopaminergic innervation [29–31]. We reasoned that octopamine release might also be triggered by olfactory signaling within the visual system to modulate Hx responses.
We first determined that the octopaminergic terminals innervating the lobula plate show increased GCaMP fluorescence in response to an odor pulse (Figure 4A), which was demonstrated in each fly tested (Figure 4B). To examine whether these octopaminergic interneurons make synapses with Hx, we made use of a genetic construct that recombines GFP between two cells in close contact (GFP reconstitution across cell-cell connections such as gap junctions between Tdc2 and Hx. In addition to implicating Tdc2 in the olfactory modulation of Hx, our GRASP data also support prior findings demonstrating that octopaminergic signaling in the brain is necessary for locomotion-induced gain in LPTCs [12].

Figure 2. Two-Photon Calcium Imaging and Characterization of Hx Tuning Properties
(A) Perspective-matched LED arena display within the imaging apparatus, equipped with an olfactometer.
(B) Posterior view confocal image of Hx dendrites within the right lobula plate via Odd-skipped-Gal4/GFP. The dashed white rectangle indicates the imaging ROI. Neuropil is indicated in purple (nc82 staining).
(C) Enlarged cross-section of lobula plate demonstrates the four layers of the lobula plate, with Hx innervation restricted to layer 2. Arrowheads indicate layer-specific directional tuning of T4T5 innervation [23].
(D) Mean responses ± SEM to a vertical bar of varying width revolving in each of two horizontal directions across the display. n = 7 animals.
(E) Directional tuning of Hx. A square-wave grating (27° wavelength) was moved in each direction as indicated on the x axis, and maximum ∆F/F was normalized to the largest response observed. Points indicate mean responses ± SEM. Red point and arrowhead indicate the stimulus direction giving maximum response, used in (F), n = 7 animals.
(F) Temporal frequency tuning of Hx. A square-wave grating was moved at constant velocity from back to front. Points indicate mean responses ± SEM. n = 7 animals.

Likely owing to the role of Odd-skipped in development, driving neuronal inactivators with Hx-Gal4 is lethal and nevertheless would have been impossible to evaluate for visual-olfactory integration due to its expression in both visual and olfactory centers [25]. Therefore, we reasoned that if octopaminergic modulation of visual circuitry is important for odor-tracking behavior, then the absence of octopaminergic signaling throughout the brain should strongly perturb odor-tracking behavior. To test this hypothesis, we used a fly strain carrying a null (loss-of-function) mutation in the Drosophila vesicular monoamine transporter (dVMAT) [13]. Rescue with a DVMAT transgene in octopaminergic neurons, but not with dopaminergic or serotonergic neurons, is sufficient to restore plume-tracking behavior (Figure 4D). As a negative control, we tested animals rescued with a DVMAT trafficking mutant (Tdc2-Gal4/Δ3VMAT [13]); these animals were unable to maintain their heading within the odor plume of the olfactory flight simulator (Figure 4D). These three lines of evidence—odor activation of Tdc2 cells, GFP puncta (GRASP) between Hx and Tdc2 neurons, and the rescue of olfactory tracking when synaptic release by octopaminergic cells is restored—provide a parsimonious interpretation that...
odor-driven octopamine release modulates the gain of visual circuitry.

Octopamine mediates locomotion-induced modulation of another LPTC, the HSE neuron [12], which is not activated by odor (Figure 3H). This provides an exciting experimental platform for broader investigation into how aminergic signaling differentially modulates postsynaptic targets within the same neuropil. It is possible that, like Hx, HSE is also modulated by odor, but that the effect is observable only when superposed with a flight-activated increase in visual response gain [33]. Additionally, like norepinephrine, octopamine acts through multiple receptor-signaling pathways having wide-ranging influences over cellular physiology. One receptor class (OCT-R) elevates intracellular cAMP levels [34] to act as either an agonist or an antagonist on synaptic and behavioral plasticity in an octopamine receptor-dependent fashion [34]. Differential receptor expression could in turn mediate differential octopaminergic neuromodulation of visual circuitry.

In summary, we have revealed a novel cellular cross-modal interaction that could support behavioral findings whereby food odor detection increases visual stability in an odor plume. Future work could elaborate additional neuronal pathways supporting related cross-modal behaviors such as enhanced salience of visual objects by odor [35]. These cross-modal interactions provide a mechanism to dynamically enhance sensory perception in a contextually appropriate manner.

Experimental Procedures

Animals
For behavior experiments, we used wild-type D. melanogaster, 3- to 6-day-old posteclosion females. Other lines used for behavior and imaging experiments included T4T5-Gal4 (Bloomington ID 40034), Tdc2-Gal4 (Bloomington ID 9313), UAS-Kir2.1-EGFP (Bloomington ID 6596), UAS-mCD8::GFP (Bloomington ID 49211), UAS-GCaMP6m (Bloomington ID 42749), UAS-GCaMP6s (Bloomington ID 42749), and Odd-Skipped-Gal4 [9]. GRASP constructs were generated using the transgenes Odd-Gal4 [9], Tdc2-LexA [38], and UAS-CD4::spGFP11; LexAop-CD4::spGFP11 [37]. Random individuals were selected from a population for each experimental group according to genotype. No experimenter blinding was done.

Behavior
Closed-Loop Magnetic-Tether Flight Simulator
The magnetic-tether flight arena allows a fly to steer freely in the yaw plane, allowing assessment of odor plume-tracking capability, and has been described in detail previously [6, 24, 38].

Rigid-Tether Flight Simulator
The rigid-tether arena records a fixed fly’s wing kinematic responses to visual stimuli, closing a feedback loop to allow the animal to control the velocity of image motion on the display or allow the assessment of visual response gain under open-loop feedback conditions, and has been described in detail previously [5, 20]. Odor was delivered through a narrow nozzle as described in detail previously [6, 24, 38].

Calcium Imaging
Adult female D. melanogaster expressing the genetically encoded calcium indicator GCamp6m [40] under one of the four Gal4 drivers were anesthetized under cold sedation. Imaging was performed with a two-photon microscope.
Figure 4. Octopaminergic Neurons Innervating the Lobula Plate Are Activated by Odor, Make Close Contact with Hx, and Are Required for Behavioral Plume Tracking

(A) Intracellular calcium dynamics (ΔF/F, GCaMP6s) of octopaminergic terminals innervating the lobula plate in response to olfactory stimulation. Asterisks indicate significance between odor off (black line) and odor on (orange line) shown above the mean ΔF/F response (two-way paired t test, p < 0.005). n = 6 animals.

(B) Mean maximum ΔF/F for each individual animal during a period preceding the odor pulse (black) and during the odor pulse (orange). Horizontal bars over the ΔF/F response in (A) indicate the measurement epochs. n = 6 animals.

(C) GFP expression by GRASP indicates octopaminergic (Tdc2-Gal4) connections with Hx (Odd-Gal4). Inset shows Hx arborization pattern to highlight similarity in GFP profile between GRASP and the lobula plate tangential cell.

(D) Mean time ± SEM spent in odor plume during the duration of the experiment (olfactory flight simulator; Figure 1C) for flies carrying a null mutation in the Drosophila vesicular monoamine transporter dVMAT rescued with either a wild-type DVMAT transgene in octopaminergic neurons (Tdc2-Gal4/VMAT) or a DVMAT trafficking mutant (Tdc2-Gal4/Δ3VMAT). Asterisk indicates significant difference (two-way paired t test, p < 0.05) between VMAT (n = 32 animals) and Δ3VMAT (n = 21 animals). Also shown is mean time in plume for Tdc2-Gal4/VMAT-rescued flies exposed to water rather than vinegar (n = 32 animals, *p < 0.05 by two-way paired t test).

References

1. Shams, L., and Kim, R. (2010). Crossmodal influences on visual perception. Phys. Life Rev. 7, 269–284.
2. Amedi, A., Malach, R., Hendler, T., Peled, S., and Zohary, E. (2001). Visuo-haptic object-related activation in the ventral visual pathway. Nat. Neurosci. 4, 324–330.
3. Watkins, S., Shams, L., Josephs, O., and Rees, G. (2007). Activity in human V1 follows multisensory perception. Neuroimage 37, 572–578.
4. Meredith, M.A., and Stein, B.E. (1986). Visual, auditory, and somatosensory convergence on cells in superior colliculus results in multisensory integration. J. Neurophysiol. 56, 640–662.
5. Chow, D.M., Theobald, J.C., and Frye, M.A. (2011). An olfactory circuit increases the fidelity of visual behavior. J. Neurosci. 31, 15035–15047.
6. Duistermars, B.J., and Frye, M.A. (2008). Crossmodal visual input for odor tracking during fly flight. Curr. Biol. 18, 270–275.
7. Frye, M.A., Tarsitano, M., and Dickinson, M.H. (2003). Odor localization requires visual feedback during free flight in Drosophila melanogaster. J. Exp. Biol. 206, 843–855.
8. Stewart, F.J., Baker, D.A., and Webb, B. (2010). A model of visual-olfactory integration for odor localisation in free-flying fruit flies. J. Exp. Biol. 213, 1886–1900.

Acknowledgments

Support for this work was provided by the National Institute of Mental Health (R01 MH076900 to D.E.K.), the Brain and Behavior Research Foundation, and the Joanne and George Miller and Family Endowed Chair in Depression Research at the UCLA Brain Research Institute (D.E.K.); the Wellcome Trust (WT085028MA to C.L.); and the Howard Hughes Medical Institute and US Air Force Office of Scientific Research (FA9550-12-1-0034) (M.A.F.).
9. Levy, P., and Larsen, C. (2013). Odd-skipped labels a group of distinct neurons associated with the mushroom body and optic lobe in the adult Drosophila brain. J. Comp. Neurol. 521, 3716–3740.

10. Krapp, H.G., Hengstenberg, B., and Hengstenberg, R. (1998). Dendritic structure and receptive-field organization of optic flow processing interneurons in the fly. J. Neurophysiol. 79, 1902–1917.

11. Schnell, B., Joesch, M., Forstner, F., Raghu, S.V., Otsuna, H., Ito, K., Borst, A., and Reiff, D.F. (2010). Processing of horizontal optic flow in three visual interneurons of the Drosophila brain. J. Neurophysiol. 103, 1646–1657.

12. Suver, M.P., Mamiya, A., and Dickinson, M.H. (2012). Octopamine neurons mediate flight-induced modulation of visual processing in Drosophila. Curr. Biol. 22, 2294–2302.

13. Grygonuk, A., Chen, A., Martin, C.A., Lawal, H.O., Fei, H., Gutierrez, G., Biedermann, T., Najibi, R., Hadi, R., Chouhan, A.K., et al. (2014). The redistribution of Drosophila vesicular monoamine transporter mutants from synaptic vesicles to large dense-core vesicles impairs amine-dependent behaviors. J. Neurosci. 34, 6924–6937.

14. Land, M.F. (1973). Head movement of flies during visually guided flight. Nature 243, 299–300.

15. Schilstra, C., and van Hateren, J.H. (1999). Blowfly kinematics and flight dynamics. J. Exp. Biol. 202, 1481–1490.

16. Schilstra, C., and van Hateren, J.H. (1998). Stabilizing gaze in flying blowflies. Nature 395, 654.

17. Hengstenberg, R. (1991). Gaze control in the blowfly Calliphora: a multisensory, two-stage integration process. Semin. Neurosci. 3, 19–29.

18. Duistermars, B.J., Care, R.A., and Frye, M.A. (2012). Binocular interactions underlying the classic optomotor responses of flying flies. Front. Behav. Neurosci. 6, 6.

19. Götz, K.G. (1968). Flight control in Drosophila by visual perception of motion. Kybernetik 4, 199–208.

20. Reiser, M.B., and Dickinson, M.H. (2008). A modular display system for insect behavioral neuroscience. J. Neurosci. Methods 167, 127–139.

21. Theobald, J.C., Ringach, D.L., and Frye, M.A. (2010). Dynamics of optomotor responses in Drosophila to perturbations in optic flow. J. Exp. Biol. 213, 1366–1375.

22. Haikala, V., Joesch, M., Borst, A., and Mauss, A.S. (2013). Optogenetic control of fly optomotor responses. J. Neurosci. 33, 13927–13934.

23. Maisak, M.S., Haag, J., Ammer, G., Serbe, E., Meier, M., Leonhardt, A., Schilling, T., Bahl, A., Rubin, G.M., Nern, A., et al. (2013). A directional tuning map of Drosophila elementary motion detectors. Nature 500, 212–216.

24. Krishnan, P., Duistermars, B.J., and Frye, M.A. (2011). Odor identity influences tracking of temporally patterned plumes in Drosophila. BMC Neurosci. 12, 62.

25. Chen, T.-W., Wardill, T.J., Sun, Y., Pulver, S.R., Renninger, S.L., Baohan, A., Schreiter, E.R., Kerr, R.A., Orger, M.B., Jayaraman, V., et al. (2013). Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499, 295–300.

26. Tuthill, J.C., Chiappe, M.E., and Reiser, M.B. (2011). Neural correlates of illusory motion perception in Drosophila. Proc. Natl. Acad. Sci. USA 108, 9685–9690.

27. Chiappe, M.E., Seelig, J.D., Reiser, M.B., and Jayaraman, V. (2010). Walking modulates speed sensitivity in Drosophila motion vision. Curr. Biol. 20, 1470–1475.

28. Jung, S.N., Borst, A., and Haag, J. (2011). Flight activity alters velocity tuning of fly motion-sensitive neurons. J. Neurosci. 31, 9231–9237.

29. van Breugel, F., Suver, M.P., and Dickinson, M.H. (2014). Octopaminergic modulation of the visual flight speed regulator of Drosophila. J. Exp. Biol. 217, 1737–1744.

30. Longden, K.D., and Krapp, H.G. (2010). Octopaminergic modulation of temporal frequency coding in an identified optic flow-processing interneuron. Front. Syst. Neurosci. 4, 153.

31. de Haan, R., Lee, Y.-J., and Nordström, K. (2012). Octopaminergic modulation of contrast sensitivity. Front Integr Neurosci 6, 55.

32. Feinberg, E.H., Vanhoven, M.K., Bendesky, A., Wang, G., Fetter, R.D., Shen, K., and Bargmann, C.I. (2008). GFP Reconstitution Across Synaptic Partners (GRASP) defines cell contacts and synapses in living nervous systems. Neuron 57, 353–363.

33. Maimon, G., Straw, A.D., and Dickinson, M.H. (2010). Active flight increases the gain of visual motion processing in Drosophila. Nat. Neurosci. 13, 393–399.

34. Farooqui, T. (2012). Review of octopamine in insect nervous systems. Open Access Insect Physiol. 4, 1–17.