UNIVERSITY OF CALIFORNIA, SAN DIEGO

# Individual glomeruli mediate innate olfactory attraction and aversion in Drosophila

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy

in

Biology

by

Julia L. Semmelhack

Committee in charge:

Professor Jing W. Wang, Chair
Professor Charles S. Zuker, Co-Chair
Professor Jeffry S. Isaacson
Professor William B. Kristan
Professor Emily R. Troemel

2009

The Dissertation of Julia Lee Semmelhack is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

---

---

---

---

Co-Chair

---

Chair

University of California, San Diego

2009

# EPIGRAPH

I should think that we might fairly gauge the future of biological science, centuries ahead, by estimating the time it will take to reach a complete, comprehensive understanding of odor. It may not seem to be a profound enough problem to dominate all the life sciences, but it contains, piece by piece, all the mysteries.

*Lewis Thomas*

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGEMENTS

Chapter 2, in part and with modifications, was published in PNAS in 2007, under the title “Propagation of olfactory information in *Drosophila*”. The dissertation author and Cory Root contributed equally to this paper. Allen Wong, Jorge Flores, and Jing Wang were co-authors.

Chapters 3 and 4 were published in Nature in 2009 under the title “Select *Drosophila* glomeruli mediate innate olfactory attraction and aversion”. The dissertation author was the primary author of this paper, with Jing Wang as co-author.

## *Curriculum Vitae*

**Julia L. Semmelhack**

### Education

Ph.D. in Biology, University of California, San Diego, September 2009
Advisor: Dr. Jing Wang

A.B. in Molecular Biology, Princeton University, June 2001
Advisor: Dr. Bonnie Bassler

### Publications

Semmelhack, J.L., and Wang, J.W. Select *Drosophila* glomeruli mediate innate olfactory attraction and aversion. *Nature.* 459, 218-224 (2009).

Root, C.M.*, Semmelhack, J.L.*, and Wang, J.W. Propagation of olfactory information in Drosophila. *Proc. Natl. Acad. Sci. USA* 104, 11826-11831 (2007). * *these authors contributed equally.*

Taga, M.E., Semmelhack, J.L., and Bassler B.L. The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in Salmonella typhimurium. *Mol. Microbiol.* 42, 777-793 (2001).

### Research Experience

Doctoral research – UC San Diego
Characterized the receptors activated by behaviorally relevant odors using two-photon imaging. Developed an assay to measure olfactory attraction and aversion in single flies. Used genetic tools to silence and restore function to receptor neurons in order to determine their role in the behavioral output.

Undergraduate Thesis Research – Princeton University
Screened for and characterized genes regulated by the autoinducer AI-2 in *Salmonella typhimurium.*

**Conference Presentations**

"Select glomeruli in the *Drosophila* antennal lobe mediate innate attraction and aversion." Synapses: from Molecules to Circuits & Behavior. Cold Spring Harbor Laboratory, April 2009. Oral presentation.

"Select glomeruli in the *Drosophila* antennal lobe mediate innate attraction and aversion." Keystone Symposium, Chemical Senses: Receptors and Circuits, March 2009. Oral presentation.

"Glomerulus-specific propagation of olfactory information in the *Drosophila* antennal lobe." Keystone Symposium, Chemical Senses: From Genes to Perception, January 2007. Poster presentation.

**Teaching Experience**

2006 Teaching Assistant, Neurophysiology Laboratory
2005 Teaching Assistant, Cellular Neurobiology
2004 Teaching Assistant, Animal Physiology Laboratory

ABSTRACT OF THE DISSERTATION

Individual glomeruli mediate innate olfactory attraction and aversion in Drosophila

by

Julia L. Semmelhack

Doctor of Philosophy

University of California, San Diego, 2009

Professor Jing W. Wang, Chair
Professor Charles S. Zuker, Co-Chair

In the *Drosophila* olfactory system, as in many other organisms, different odorants activate specific patterns of glomeruli, but the mechanisms by which these patterns are transformed and eventually read out to lead to behavior remain unknown. One outstanding question is the degree to which the responses of the second order projection neurons are derived from direct receptor input, as opposed to interneurons that receive input from multiple glomeruli. Using mutants for two receptors in conjunction with PN imaging, we found that the cognate receptor neurons are the main driver of PN responses. To address the question of how glomerular patterns elicit behavior, we used

genetic tools to dissect the contribution of each glomerulus that is activated by the food odorant apple cider vinegar. This odorant triggers robust innate attraction at a low concentration, which activates six glomeruli. We found that two of these glomeruli were necessary and sufficient for this behavior. At a higher concentration, apple cider vinegar becomes markedly less attractive. We found that one glomerulus, which is activated by the higher concentration, was responsible for the switch in behavior. These results show that innate attraction and repulsion to a food odorant is mediated by individual glomeruli rather than the combination of activated glomeruli.

# Chapter 1

All organisms must detect important features of their external world, and create an internal representation of that information which is translated into a behavioral response. The representation is far from complete; perception is attuned to those features of the environment that are vital for survival and reproduction. How this is accomplished is a fundamental problem in systems neuroscience.

Smell is perhaps the most evolutionarily ancient of the senses, and the system's basic organization has been remarkably conserved in organisms from flies to humans. Many animals rely on it for the essential functions of finding food and mates and avoiding predators, and even humans, which are relatively less dependent on olfaction, can identify over 10,000 different odorants. Despite this, the organization of the olfactory system remained poorly understood until quite recently, perhaps because olfaction is unlike the visual, somatosensory, and auditory systems in that it does not extract spatial information about the stimulus, and thus can use spatial parameters to encode the quality of an odor. Olfaction could be considered simpler in the sense of having fewer features to encode than for example the visual system, which must represent orientation, motion direction, speed, color, etc. But, odor identity, the key feature that the system must encode, is not organized along a continuous spectrum, which is perhaps why the basic organization of the system remained mysterious for a relatively long time.

Unlike other sensory systems, traditional methods of electrophysiology and anatomy failed to reveal a topographical organization in the olfactory bulb. The first indications of the spatial organization of the olfactory system were provided by 2-deoxyglucose imaging studies in the rat. An analysis of the patterns of activity elicited by different odors revealed that distinct but overlapping sets of glomeruli were labeled[1].

Furthermore, as the concentration of the odorant was increased, the number of labeled foci grew larger. These results suggested that odor identity and concentration could be encoded by the spatial pattern of activity in the bulb. A high-resolution study of 2-deoxyglucose labeling revealed that the labeled foci probably corresponded to glomeruli, and that the neuropil within a given glomerulus tended to show a uniform level of activity[2]. This suggested that the glomerulus could constitute a functional unit.

The discovery of the multigene family of receptors [3] served to clarify the underlying logic of the system by showing that odorants are recognized by a large and diverse family of genes, and opened the door to subsequent molecular dissections of olfaction. Probing the rat olfactory bulb for receptor mRNA revealed each individual receptor probe labeled one glomerulus, suggesting that each glomerulus receives input from sensory neurons expressing one particular receptor[4]. Furthermore, a subsequent study created mice expressing a marker under control of an OR promoter, and found that each class of ORNs projects to a particular, spatially invariant glomerulus[5]. Thus, the question of which receptors are activated by a given odor can be answered by observing the combination of active glomeruli. Since rodents have roughly 1000 receptor genes but can distinguish far more than 1000 different odors, it was presumed that odor identity would have to be encoded combinatorially. Indeed, when individual mouse ORNs were subjected to calcium imaging followed by OR gene sequencing, it was found that most of them responded to multiple odorants, and that most of the odorants were recognized by multiple receptors[6]. Thus, different odorants may be encoded by the combination of receptors and glomeruli that they activate.

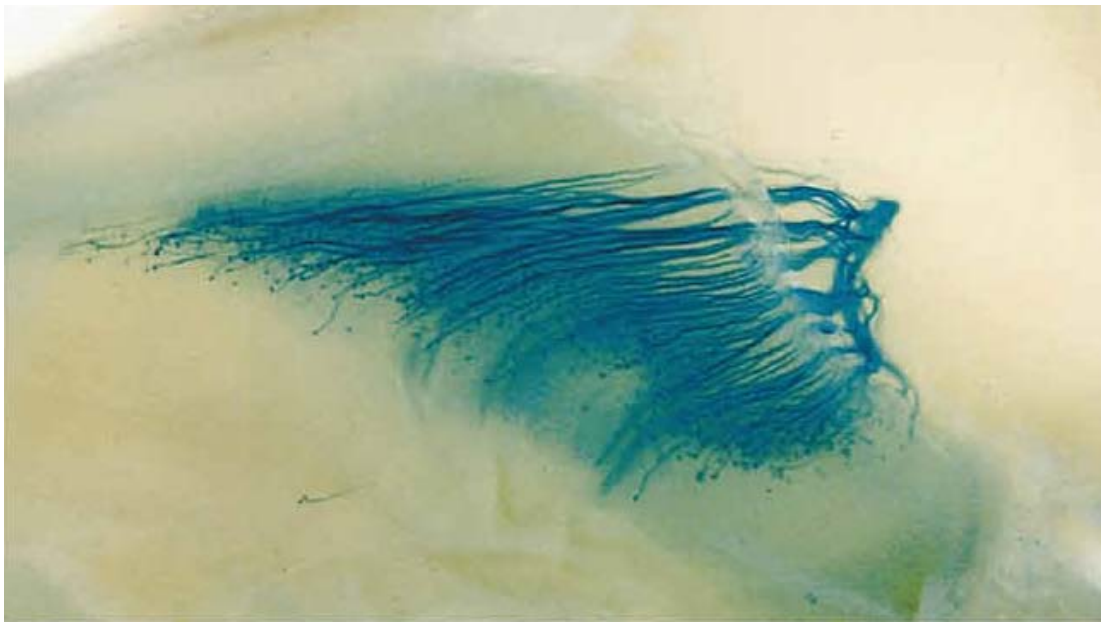


**Figure 1. Axons of ORNs expressing one receptor converge on one glomerulus in the mouse olfactory bulb.** Whole mount of the nasal cavity and medial olfactory bulb in a mouse with the *LacZ* transgene expressed under control of the P2 receptor promoter. From [5].

If odor identity is encoded by the combination of activated glomeruli, signals from different glomeruli must be integrated at some point. In the mouse, one possible locus of integration is the olfactory cortex, where pyramidal neurons receive input from mitral cells. This model was tested by comparing the responses of olfactory cortex pyramidal neurons to mixtures and their components[7]. Many pyramidal neurons that did not respond to single components were activated by the mixture, indicating that these neurons get input from multiple glomeruli, and could encode the combination of glomeruli.

Despite the progress made towards understanding the molecular logic of olfaction in rodents, outstanding questions remain about how receptor activation is read out by the brain and converted to behavioral output. Some of these questions have begun to be answered in the olfactory system of *Drosophila.* The fruit fly provides an attractive model system because while the basic organization of the system is intact, it is numerically simpler than its vertebrate counterpart. There are approximately 60 glomeruli and receptors. Olfactory receptor neurons (ORNs) are housed in the two olfactory organs, the antenna and the maxillary palp. Each ORN expresses one or a few receptors, and, as in vertebrates, ORNs that express the same receptor all project to a single glomerulus[8].

One of the advantages of the fly is that genetically encoded reporters of neuronal activity can be expressed in defined populations of neurons for functional imaging. Responses to odors in the antennal lobe have been recorded using GCaMP to show that different odors evoke distinct patterns of active glomeruli, and that these

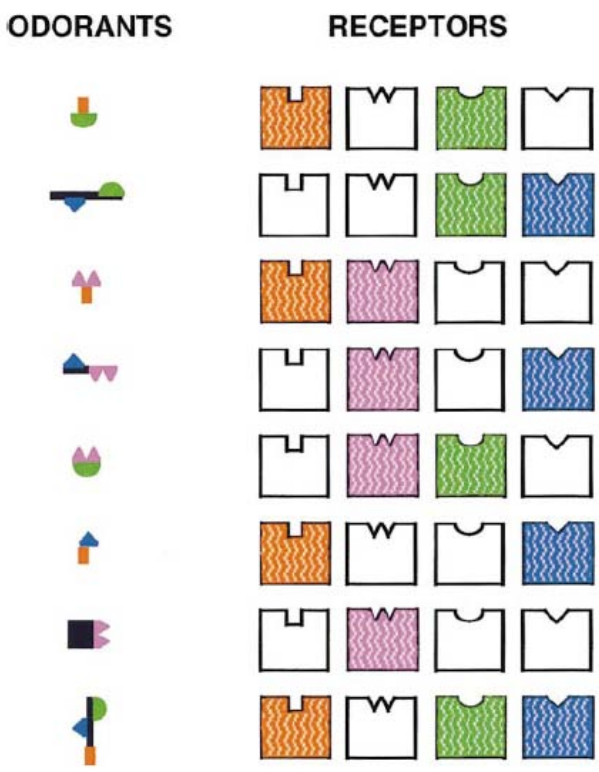



**Figure 2. Combinatorial receptor codes for odorants.** Model for how the identities of various odorants could be encoded by combinations of receptors. From[6].

patterns are conserved from one fly to another, and that increasing the odorant concentration increases the number of active glomeruli[9]. Another group used synapto-pHluorin to investigate information processing within the antennal lobe, showing that ORN and PN responses are quite similar, while inhibitory local neurons are more broadly tuned[10].

As in the mouse, a key question in *Drosophila* olfaction was how the neurons that get input from each glomerulus (projection neurons, or PNs) project to the next brain areas, the lateral horn and mushroom body. Is the stereotyped organization of the antennal lobe preserved, or are receptor channels mixed? This question was investigated by labeling single PNs and examining their axonal projection patterns[11, 12]. Projection neurons that innervate a particular glomerulus have a stereotyped branching pattern in the lateral horn, and that pattern overlaps with that of PNs from other glomeruli. Thus, the neurons in this brain region could potentially integrate inputs from multiple glomeruli.

Although anatomical and functional data have suggested that the olfactory system could operate in a combinatorial fashion, the question of how the patterns of active glomeruli are read out must be answered by behavioral experiments. Recently, substantial progress has been made towards elucidating the links between glomerular activation and behavior. One such study investigated the encoding of social cues present in mouse urine[13]. The authors used gas chromatography in conjunction with single-unit recording to investigate how mitral cells represent the cues unique to male urine. A small subset of mitral cells responded to one component of male urine, which was identified as (methylthio)methanethiol (MTMT). Remarkably, the addition of MTMT to castrated male urine was sufficient to enhance the attractiveness of the urine for female mice.

These findings are an example of certain glomeruli acting as specialized feature detectors for an ecologically relevant stimulus. However, this paper did not ask whether the MTMT-responsive glomerulus or glomeruli that they were recording from were responsible for the behavior, due to the difficulty of identifying and manipulating glomeruli in this system.

In the fruit fly, far more precise manipulations of the olfactory system are possible, including both gain and loss of function experiments. One group has used these tools to address the role of pheromone-responsive glomeruli in mating behavior. *cis*-vaccenyl acetate (cVA) is a fatty-acid derivative that is produced only by males. It is thought to promote courtship receptivity in females, and to inhibit courtship in males, which prevents them from courting other males and recently mated females. A recent study investigated the role of the Or67d ORNs, which respond to cVA, in mating behavior. *Or67d* mutant male flies showed increased courtship of other males, and mutant females had a much longer latency to copulation than wild type females, indicating that these ORNs are necessary for male and female courtship behavior[14]. Remarkably, when the *Bombyx mori* pheromone receptor was expressed in Or67d ORNs, its ligand, bombykol, was able to cause courtship suppression in males. The Or67d receptor channel thus constitutes a labeled line for denoting the presence of a pheromone.

The fly's response to $CO_2$ is similarly mediated by a dedicated receptor channel. $CO_2$ is an aversive stimulus at all concentrations that have been tested in flies[15], but its ecological role is not entirely clear. It has been identified as one component of the "*Drosophila* stress odorant", which is released by stressed flies, and may serve as a stress or threat signal[15]. $CO_2$ is also emitted by unripe fruit, which is a less desirable food

source than rotting fruit for *Drosophila*, and thus it could be involved in selection of food or oviposition sites[16]. In any case, $CO_2$ is an extremely aversive stimulus, which activates the V glomerulus. Silencing the V glomerulus blocks the avoidance response, and expressing channelrhodopsin in its receptor neurons causes flies to avoid blue light. This behavior, like the cVA response and, in mice, the responses to male urine, predator, and spoiled food odors, is an example of a highly ethologically relevant response that is mediated by a labeled line.

These studies of $CO_2$, cVA and MTMT concern behaviors that are critical for the organism's survival or reproduction. There are several advantages to this approach. From a practical standpoint, behaviors that are evolutionarily selected for tend to be quite robust and reliable, and thus easier to measure. Furthermore, it is often advantageous to investigate a system in light of a stimulus it evolved to detect, because sensory systems may be specialized to detect ecologically relevant stimuli. For example, the auditory system of bats has a disproportionately large representation of the frequencies the bats use for navigation[17]. While bats can hear other frequencies, any attempt to study the system that did not focus on the navigation frequencies would fail to uncover the full richness of the bat's auditory world. Similarly, the auditory system of many songbirds contains areas that respond selectively to species-specific songs, and not to simpler stimuli such as pure tones[18]. Because many birds use song for important functions such as finding mates and warning of danger, their auditory systems are attuned to the complex spectral and temporal properties of conspecific songs.

While the olfactory system detects many ecologically relevant stimuli that are important for mating, finding food and avoiding predators, most animals can also be

trained to respond to odorants that are not found in nature. How are these learned olfactory behaviors encoded? A recent study of the mouse olfactory system created mice in which large areas of the olfactory bulb were genetically ablated to ask what the roles of these areas might be for innate and learned behaviors[19]. Kobayakawa *et. al.* generated one strain of mice that expressed the diphtheria toxin gene in class I and some class II ORNs, leaving the antero-dorsal half of the olfactory bulb vacant. These ΔD mice show specific defects for innate avoidance of two stimuli, 2-methylbutyric (2MB) acid, which is associated with spoiled food, and trimethyl-thiazoline (TMT), which is found in fox feces. Normally, TMT induces a freezing response in mice, and activates a specialized pathway that increases the level of stress hormone. In ΔD mice, TMT fails to induce the elevation of stress hormone, suggesting that the innate fear response is mediated by one of the ablated glomeruli. Interestingly, the ΔD mice can be conditioned to avoid both 2MB and TMT. All together, these results indicate that certain glomeruli in particular zones of the mouse olfactory bulb are hardwired for innate behaviors, while others that are normally neutral can be associated with an aversive response after training.

The notion that certain receptors are hardwired for specific behaviors while others can be associated with a given behavior through training is consistent with studies showing that large areas of the rat olfactory bulb can be ablated with little or no effect on odor discrimination[20, 21]. In one experiment, the area of the bulb that is strongly activated by a particular odorants was ablated, and the rat's ability to learn to discriminate that odorant from others was tested[22]. In another study, large areas of the bulb were lesioned, and after recovery, the rats were trained on a number of odors[21]. Surprisingly, the lesions did not affect olfactory discrimination except on difficult

mixture tasks. These results suggest that odor learning is not dependent on any specific glomeruli. Thus, an animal with most of its bulb missing can still learn to recognize an odor if it has a glomerulus that responds to that odor intact. A more recent paper genetically perturbed 95% of all ORNs such that they expressed one odorant receptor[23]. These mice showed only minor deficits on most odor learning tasks, suggesting that the remaining 5% of the glomeruli were sufficient to drive learned behavior. In contrast, the animal's ability to perform innate olfactory behaviors was dramatically affected. Male mice showed a reduced frequency of mating and aggressive behaviors, indicating that these behaviors require input from ORNs that are now expressing the transgenic receptor. Thus, while the two innate behaviors that were tested were impaired, olfactory learning was more flexible, and could be achieved with the limited set of remaining glomeruli.

A recent study in flies supports the idea that olfactory learning is flexible and requires minimal glomeruli. *Or83b* null flies fail to learn to associate electric shock with most odorants. But, when *Or83b* was restored in a single class of ORNs, that one glomerulus proved to be sufficient to drive learned behavior for odors that activated it[24].

As described in this thesis, innate attraction and repulsion to a food odorant in *Drosophila* are mediated by only a few glomeruli out of the larger set of activated glomeruli. As was the case for other ecologically relevant stimuli, the response to cider vinegar is elicited by certain glomeruli that serve as labeled lines attractive and aversive behavior. The fact that not all of the activated glomeruli are required for the response raises the question of what the function of the other glomeruli might be. Based on the

studies in flies and rodents mentioned above, it seems likely that the fly could use these glomeruli for learned olfactory behavior.

**References**


1. Stewart, W.B., J.S. Kauer, and G.M. Shepherd, *Functional organization of rat olfactory bulb analysed by the 2-deoxyglucose method.* J Comp Neurol, 1979. **185**(4): p. 715-34.

2. Lancet, D., et al., *Mapping of odor-related neuronal activity in the olfactory bulb by high-resolution 2-deoxyglucose autoradiography.* Proc Natl Acad Sci U S A, 1982. **79**(2): p. 670-4.

3. Buck, L. and R. Axel, *A novel multigene family may encode odorant receptors: a molecular basis for odor recognition.* Cell, 1991. **65**(1): p. 175-87.

4. Vassar, R., et al., *Topographic organization of sensory projections to the olfactory bulb.* Cell, 1994. **79**(6): p. 981-91.

5. Mombaerts, P., et al., *Visualizing an olfactory sensory map.* Cell, 1996. **87**(4): p. 675-86.

6. Malnic, B., et al., *Combinatorial receptor codes for odors.* Cell, 1999. **96**(5): p. 713-23.

7. Zou, Z. and L.B. Buck, *Combinatorial effects of odorant mixes in olfactory cortex.* Science, 2006. **311**(5766): p. 1477-81.

8. Vosshall, L.B., A.M. Wong, and R. Axel, *An olfactory sensory map in the fly brain.* Cell, 2000. **102**(2): p. 147-59.

9. Wang, J.W., et al., *Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain.* Cell, 2003. **112**(2): p. 271-82.

10. Ng, M., et al., *Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly.* Neuron, 2002. **36**(3): p. 463-74.

11. Wong, A.M., J.W. Wang, and R. Axel, *Spatial representation of the glomerular map in the Drosophila protocerebrum.* Cell, 2002. **109**(2): p. 229-41.

12. Marin, E.C., et al., *Representation of the glomerular olfactory map in the Drosophila brain.* Cell, 2002. **109**(2): p. 243-55.

13. Lin, D.Y., et al., *Encoding social signals in the mouse main olfactory bulb.* Nature, 2005. **434**(7032): p. 470-7.

14. Kurtovic, A., A. Widmer, and B.J. Dickson, *A single class of olfactory neurons mediates behavioural responses to a Drosophila sex pheromone.* Nature, 2007. **446**(7135): p. 542-6.

15. Suh, G.S., et al., *A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila.* Nature, 2004. **431**(7010): p. 854-9.

16. Faucher, C., et al., *Behavioral responses of Drosophila to biogenic levels of carbon dioxide depend on life-stage, sex and olfactory context.* J Exp Biol, 2006. **209**(Pt 14): p. 2739-48.

17. Covey, E., *Neurobiological specializations in echolocating bats.* Anat Rec A Discov Mol Cell Evol Biol, 2005. **287**(1): p. 1103-16.

18. Cousillas, H., et al., *Functional organization of the forebrain auditory centres of the European starling: a study based on natural sounds.* Hear Res, 2005. **207**(1-2): p. 10-21.

19. Kobayakawa, K., et al., *Innate versus learned odour processing in the mouse olfactory bulb.* Nature, 2007. **450**(7169): p. 503-8.

20. Lu, X.C. and B.M. Slotnick, *Recognition of propionic acid vapor after removal of the olfactory bulb area associated with high 2-DG uptake.* Brain Res, 1994. **639**(1): p. 26-32.

21. Lu, X.C. and B.M. Slotnick, *Olfaction in rats with extensive lesions of the olfactory bulbs: implications for odor coding.* Neuroscience, 1998. **84**(3): p. 849-66.

22. Slotnick, B.M., et al., *Detection of propionic acid vapor by rats with lesions of olfactory bulb areas associated with high 2-DG uptake.* Brain Res, 1987. **417**(2): p. 343-6.

23. Fleischmann, A., et al., *Mice with a "monoclonal nose": perturbations in an olfactory map impair odor discrimination.* Neuron, 2008. **60**(6): p. 1068-81.

24. DasGupta, S. and S. Waddell, *Learned odor discrimination in Drosophila without combinatorial odor maps in the antennal lobe.* Curr Biol, 2008. **18**(21): p. 1668-74.

# Chapter 2

## 2.1 Abstract

Investigating how information propagates between layers in the olfactory system is an important step toward understanding the olfactory code. The second order projection neurons (PNs) of the antennal lobe receive two sources of input; the ORNs of the same glomerulus and interneurons that innervate many glomeruli. In order to determine the roles of excitatory interneurons and direct ORN input for PN activation, we used mutants for two ORs to abolish direct ORN input to two glomeruli, and examined the effect on PN odor responses. Using calcium imaging, we found that the mutants showed no odor evoked activity in the cognate PNs. Thus, the main source of excitatory input to the PNs if from the cognate ORNs.

## 2.2 Introduction

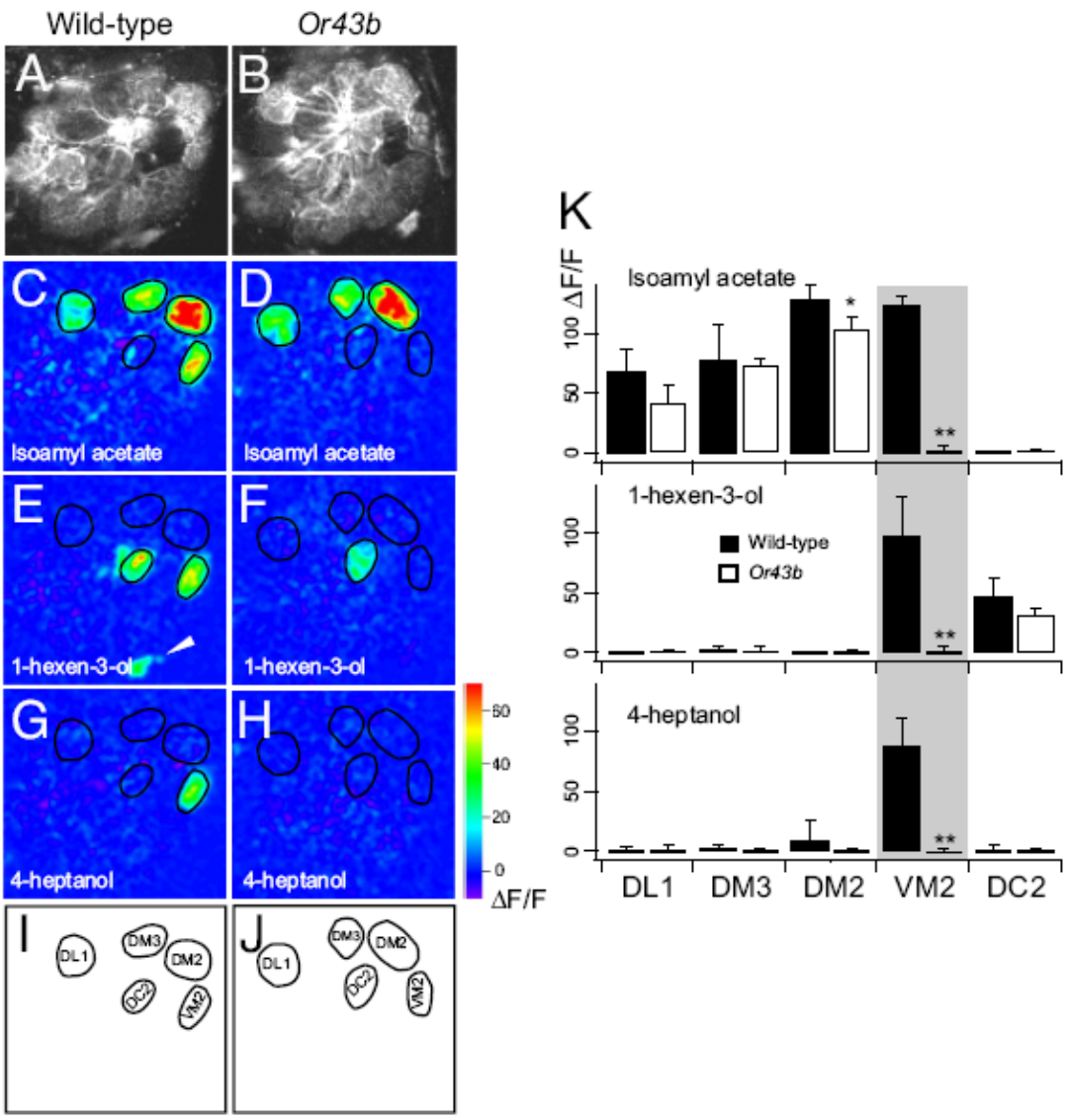
The *Drosophila* the olfactory system exhibits a highly stereotyped organization, with each glomerulus getting direct input from one type ORN, and each PN innervating one glomeulus. However, the antennal lobe contains many GABAergic inhibitory interneurons[1-3], as well as a group of recently identified cholinergic excitatory interneurons[4]. These classes of interneurons should have opposing effects on PNs, but the extent to which they contribute to PN output is unknown. It is possible that the interglomerular excitatory connections merely modulate PN responses to odors. In this case, the main source of PN excitation comes form the ORNs of the same glomerulus (its cognate ORNs). In another scenario, lateral and cognate receptor inputs constitute two independent sources of strong excitatory input for each PN. A powerful lateral input would give rise to a distributed representation of olfactory information.

To discriminate between these two models, it is necessary to dissect the relative contributions of ORNs and interneurons to PN output. Here we use OR mutant flies to ask what input PNs receive in the absence of activity in their cognate ORNs.

**2.3 Imaging PNs with no direct ORN input**

We investigated PN responses in the VM2 glomerulus in flies with a targeted mutation in its cognate ORN, *Or43b.* In order to monitor PN activity, we imaged flies bearing the *GH146-Gal4* and *UAS-GCaMP* transgenes. Figure 1 shows the typical response to odor stimulation in one optical plane of the antennal lobe. In wild-type flies, 4-heptanol, 1-hexen-3-ol, and isoamyl acetate each excite one, two, and four glomeruli in this plane (Fig. 3c, e, and g). The VM2 glomerulus of the *Or43b* mutant did not show any detectable change of intracellular calcium in response to these three different odorants (Fig. 3d, f, and h). The other glomeruli in this plane, DL1, DM3, DM2, and DC2, showed little or no reduction in odor response in the mutant flies compared to wild-type flies. These results suggest that the cognate ORNs make a much greater contribution to PN activity than interglomerular connections do.

Can these conclusions be expanded to other glomeruli? The DM1 glomerulus is innervated by ORNs that express *Or42b*, so we used a fly line with a *P*-element insertion in the *Or42b* gene to ask whether DM1 could be activated in the absence of direct ORN input. In wild-type flies, 3-octanone, benzyl acetate, and isoamyl acetate each evoked activity in the glomeruli of the plane containing DM1 (Fig 4c, e, and g). The DM1 glomerulus in the *Or42b* mutant flies did not show any detectable response to these




**Figrure 3. *Or43b* mutation eliminates the response in VM2 PNs. a, b,** Prestimulation images show glomerular structure. **c-h,** Responses to the three different odors in the wild type and the *Or43b* mutant. Odor concentration = 8% SV. **k,** statistical analysis of glomerular response. The olfactory responses in five different glomeruli were compared between the wild type and the *Or43b* mutant. *n*=4. *, $p < 0.05$; **, $p < 0.01$.

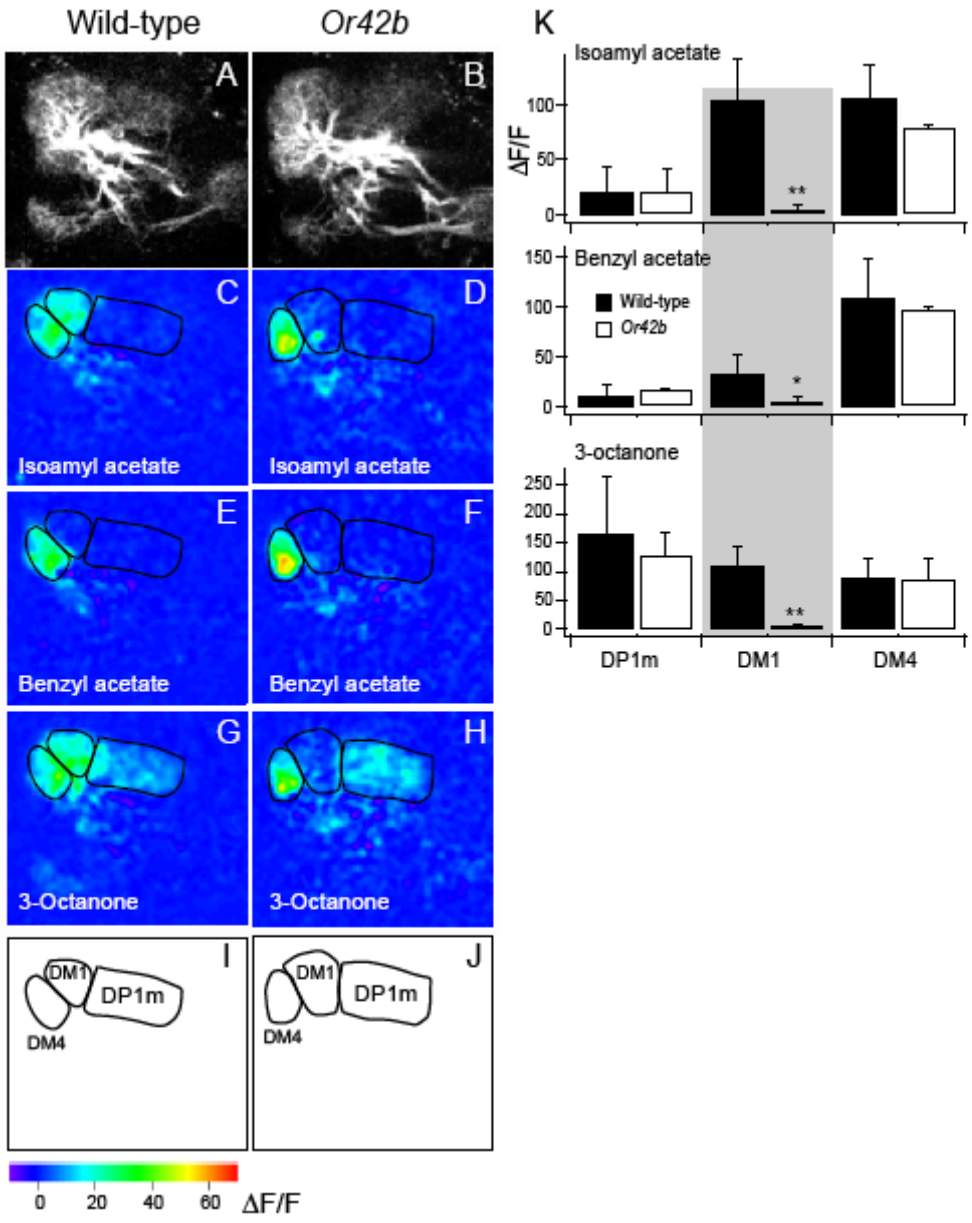



**Figure 4. The Or42b mutation eliminates odor-evoked calcium activity in the cognate DM1 projection neurons**. **a, b,** Prestimulation images show glomerular structure. **c-h,** Glomerular responses to the three different odors in the wild type and the *Or42b* mutant. Odor concentration = 8% SV. (K) Statistical analysis of glomerular response. The olfactory responses in three different glomeruli (DP1m, DM1, and DM4) were compared between the wild-type and the *Or42b* mutant. The mutant causes significant change only in the DM1 PNs (*, $P < 0.05$; **, $P < 0.01$). ΔF/F values are presented as mean ± SD, n = 6.
[5]

three different odorants (Fig 4d, f, and h).

**2.4 Discussion**

Here, we found that receptor inputs constitute the main driving force for PN excitation. What, then, are the functions of lateral interactions in the antennal lobe? Lateral inhibition has been proposed as a mechanism for olfactory processing, either by sharpening odor tuning[6, 7] or by generating synchrony[8, 9]. One plausible function of lateral excitation, in concert with lateral inhibition, is to enhance PN synchrony. Synchronized activity could facilitate the readout of the combinatorial code by the third-order neurons[10]. Another possible function could be to regulate PN sensitivity, which would allow the system to detect odors over a wide range of concentrations.

**2.5 Methods**

Flies were raised on standard medium at 22-25°C. Odorants were obtained from Sigma-Aldrich. The preparation for calcium imaging has been previously described[5]. The following transgenic fly lines were used; *UAS-GCaMP[5], GH146-Gal4[11], Or42b[12]* and *Or42b[13]* mutants.

This chapter, in part and with modifications, was published in PNAS in 2007, under the title "Propagation of olfactory information in *Drosophila*". The dissertation author and Cory Root contributed equally to this paper. Allen Wong, Jorge Flores, and Jing Wang were co-authors.

**References**


1. Wilson, R.I., G.C. Turner, and G. Laurent, *Transformation of olfactory representations in the Drosophila antennal lobe.* Science, 2004. **303**(5656): p. 366-70.

2. Wilson, R.I. and G. Laurent, *Role of GABAergic inhibition in shaping odor-evoked spatiotemporal patterns in the Drosophila antennal lobe.* J Neurosci, 2005. **25**(40): p. 9069-79.

3. Stocker, R.F., *The organization of the chemosensory system in Drosophila melanogaster: a review.* Cell Tissue Res, 1994. **275**(1): p. 3-26.

4. Shang, Y., et al., *Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe.* Cell, 2007. **128**(3): p. 601-12.

5. Wang, J.W., et al., *Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain.* Cell, 2003. **112**(2): p. 271-82.

6. Yokoi, M., K. Mori, and S. Nakanishi, *Refinement of odor molecule tuning by dendrodendritic synaptic inhibition in the olfactory bulb.* Proc Natl Acad Sci U S A, 1995. **92**(8): p. 3371-5.

7. Sachse, S. and C.G. Galizia, *Role of inhibition for temporal and spatial odor representation in olfactory output neurons: a calcium imaging study.* J Neurophysiol, 2002. **87**(2): p. 1106-17.

8. Bazhenov, M., et al., *Model of transient oscillatory synchronization in the locust antennal lobe.* Neuron, 2001. **30**(2): p. 553-67.

9. Lei, H., T.A. Christensen, and J.G. Hildebrand, *Local inhibition modulates odor-evoked synchronization of glomerulus-specific output neurons.* Nat Neurosci, 2002. **5**(6): p. 557-65.

10. Gelperin, A., *Oscillatory dynamics and information processing in olfactory systems.* J Exp Biol, 1999. **202 (Pt 14)**: p. 1855-64.

11. Stocker, R.F., et al., *Neuroblast ablation in Drosophila P[GAL4] lines reveals origins of olfactory interneurons.* J Neurobiol, 1997. **32**(5): p. 443-56.

12. Bellen, H.J., et al., *The BDGP gene disruption project: single transposon insertions associated with 40% of Drosophila genes.* Genetics, 2004. **167**(2): p. 761-81.

13. Elmore, T., et al., *Targeted mutation of a Drosophila odor receptor defines receptor requirement in a novel class of sensillum.* J Neurosci, 2003. **23**(30): p. 9906-12.

# Chapter 3

## 3.1 Abstract

Fruit flies exhibit robust attraction to food odors, which usually excite multiple glomeruli. To understand how the representation of such odors leads to behavior, we used genetic tools to dissect the contribution of each activated glomerulus. Apple cider vinegar triggers robust innate attraction at a relatively low concentration, which activates six glomeruli. By silencing individual glomeruli, we found that the absence of activity in two glomeruli, DM1 and VA2, markedly reduced attraction. Conversely, the selective activation of these two glomeruli was sufficient for attraction.

## 3.2 Introduction

A single odorant typically activates multiple receptor types [1, 2], and therefore elicits a distinct spatial pattern of activated glomeruli in the antennal lobe [3-5]. However, the mechanism by which these patterns are actually used to drive behavioral responses remains to be determined. It is possible that the whole pattern is necessary to elicit behavioral output. Alternatively, parts of the pattern, or even individual glomeruli, could be important for olfactory behaviors. This information from the antennal lobe can then be read out by higher brain centers, which probably integrate information from multiple sensory modalities to generate motor responses.

In contrast to the patterns of several glomeruli activated by most odorants, recent studies have identified two odorants that activate single glomeruli – $CO_2$ and the male-specific pheromone, cVA – which trigger innate avoidance and female courtship receptivity, respectively [6-10]. By manipulating activity in the cognate receptor neurons, the activation of these single ORN channels was shown to be necessary and sufficient to

produce the behavior, suggesting that these receptors are hardwired to specific behavioral outputs [6, 7, 11]. These examples could be special cases because these odorants activate only one glomerulus, whereas most odorants excite multiple glomeruli. Furthermore, food odors contain many individual odorants [12], thus activating multiple glomeruli. Here we set out to study innate attraction to cider vinegar, a complex and highly attractive food odor, and to determine the role of individual glomeruli within the odor evoked pattern. Fruit flies are highly attracted to vinegar, as it is associated with their favorite food source – rotting fruit [13].

### 3.3 Behavioral assay

In order to observe innate attraction behavior in individual flies, we used a four-field olfactometer design, which was recently applied to *Drosophila* [10]. By recording the outcome of multiple decisions in each fly, we were able to obtain a robust and reliable score even when using a relatively small number of flies. We measured attraction by observing single flies walking in a four-field arena, in which each quadrant received a separate air stream. When vinegar was added to one of the air streams, the fly spent most of its time in the corresponding quadrant (Fig. 6a). We recorded the location of the fly at one-second intervals, and calculated a performance index by measuring the time spent in the odor quadrant. A fly that remained in the odor quadrant for the length of the assay would receive a score of 100%, whereas a fly that distributed its time equally among the four quadrants would score 0%, and a fly that spent no time in the odor quadrant would receive a score of -100%. We found that attraction was dependent on starvation state, such that performance indices increased markedly after flies were starved more than 48

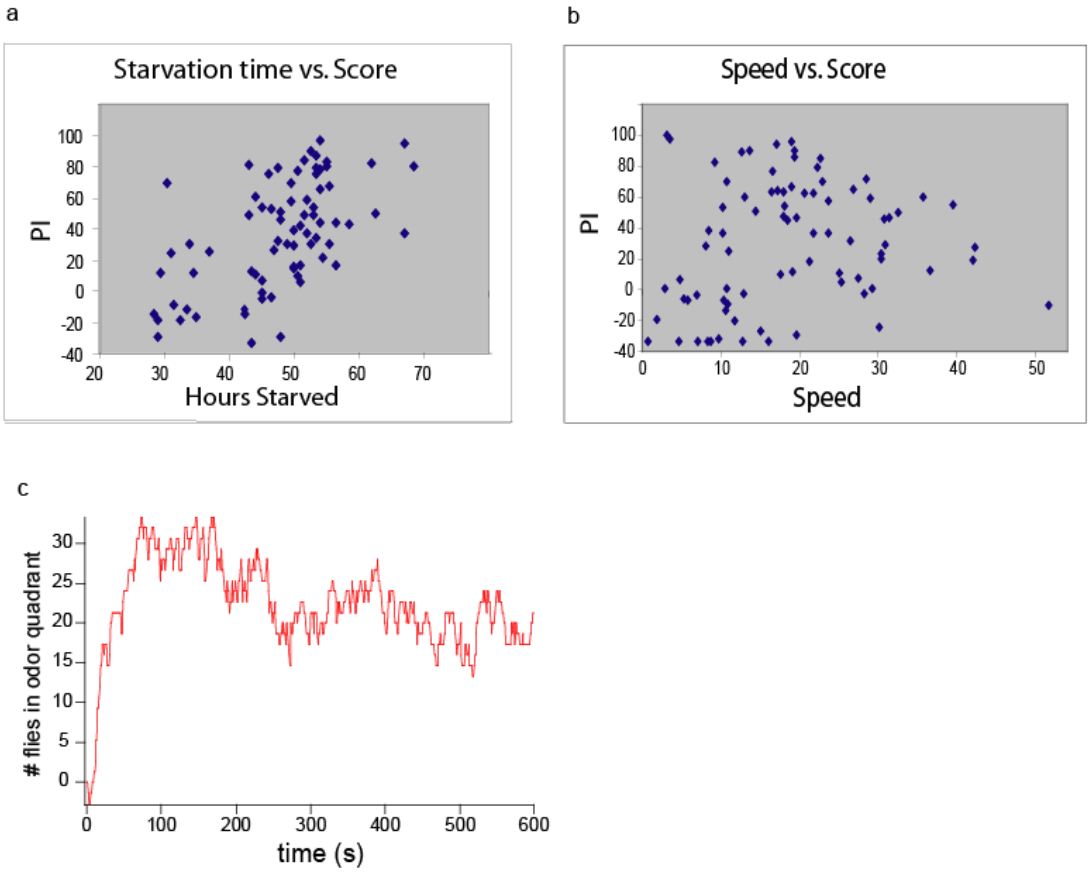



**Figure 5. Optimization of parameters for behavioral assay a,** Performance index gradually increased as flies were starved. **b,** Average attraction scores were highest in the range of speeds from 15 to 30 units/second, which translates to .5-1 cm/second. **c,** The largest number of flies were in the odor quadrant (out of 42 total) during the interval from 50-250 seconds.

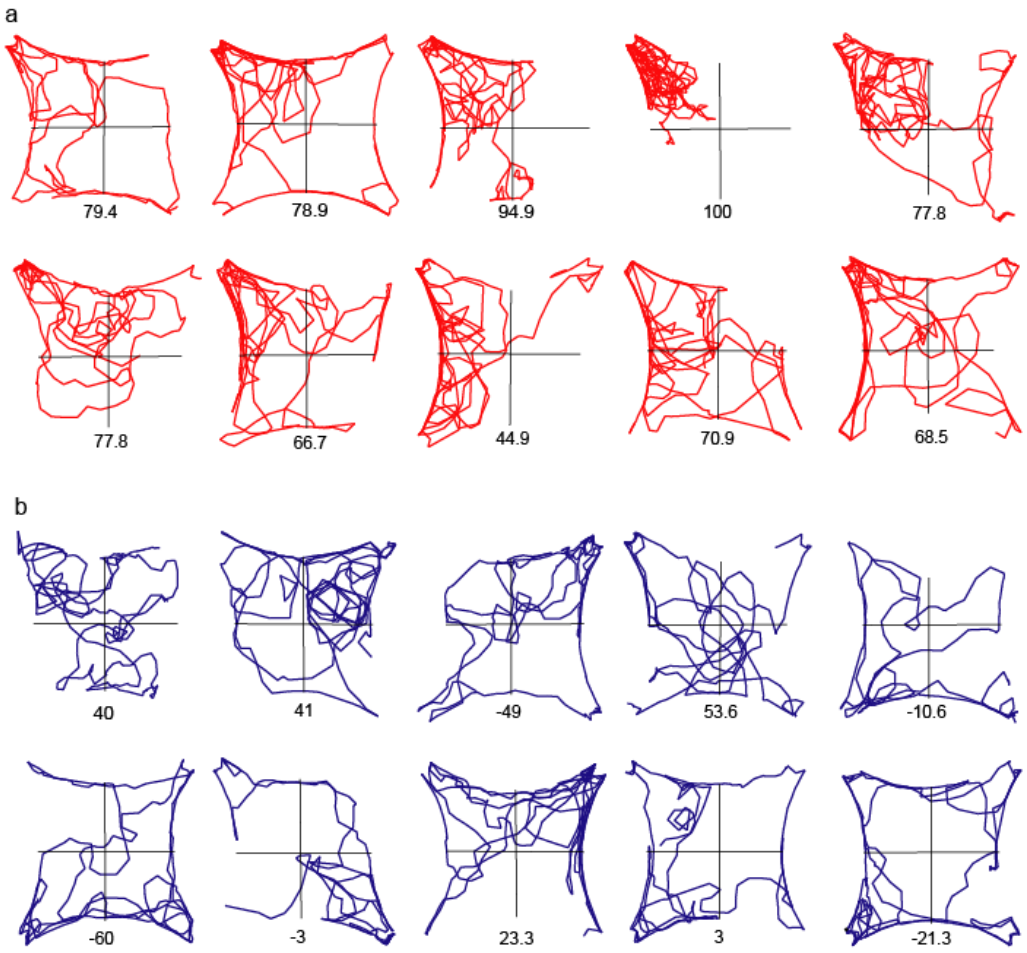



**Figure 6. Tracks of individual flies. a,** 10 $w^{1118}$ flies, with performance indices listed below. 3 ppm vinegar was delivered to the upper left quadrant. **b,** Behavioral response of 10 $Or83b^{-/-}$ flies to 3 ppm vinegar.

hours (Fig. 5a). We also found that the initial speed of the flies was somewhat predictive of their final score. Flies with a low average speed often failed to explore all quadrants of the chamber, resulting in low scores. Conversely, flies on the high end of the speed distribution tended to leave the odor quadrant after a relatively short period of time (Fig. 5b). Thus, we chose to use only flies with an initial average speed of .5-1 cm/second. Typically, flies took up to a minute to find the odor quadrant, and spent about 200 seconds in the odor quadrant before leaving to explore other parts of the arena (Fig 5c). Thus, we used the interval from 50 to 250 seconds to calculate the score.

Using a concentration of 3 ppm (isobutylene equivalents) vinegar, we saw an average performance index of 75% (Fig. 7c), which is consistent with previous results [10]. In order to verify that the behavior is mediated by the olfactory system, we measured attraction in flies whose antennae had been amputated, and found that they were indifferent to vinegar (PI = -6.7%, n=20). In addition, we tested flies with a targeted mutation of *Or83b*. *Or83b* is expressed in 80% of all ORNs [14], and acts in concert with other olfactory receptors (ORs) to generate responses to odorants [15-17]. We found that attraction was virtually abolished in *Or83b* mutant flies (Fig. 6b and Fig 7b), with the distribution of control $w^{1118}$ flies almost entirely separated from the *Or83b* mutant animals (Fig. 7c). In the absence of odors, control and mutant flies are distributed equally in all four quadrants (Fig 8). *Or83b* mutant flies showed no impairment in $CO_2$ avoidance (PI = -87 ± 9%, n=12), suggesting that their locomotion capability is normal. Thus, attraction in this assay requires ORNs, and the *Or83b* mutation provides a useful tool to link ORN activity with behavioral output.

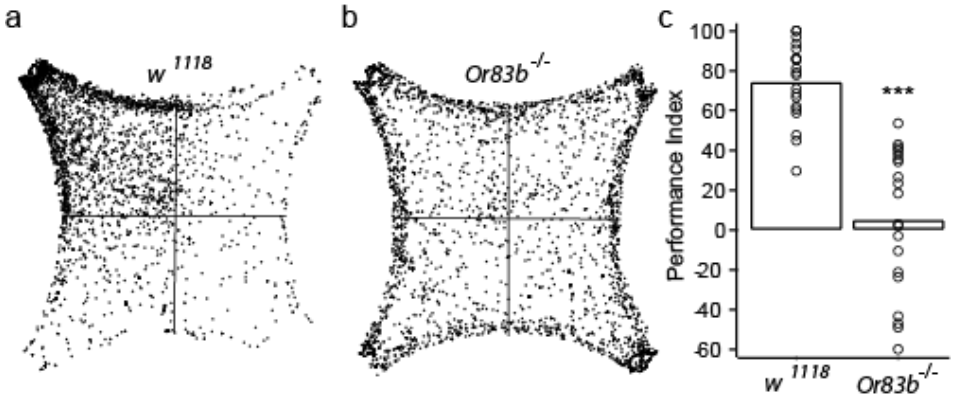



**Figure 7. Flies are robustly attracted to apple cider vinegar. a, b,** Density plot of 20 $w^{1118}$ and $Or83b^{-/-}$ flies. **d,** Performance index of $w^{1118}$ and $Or83b^{-/-}$ flies. *** indicates $P < 0.001$; T-test.

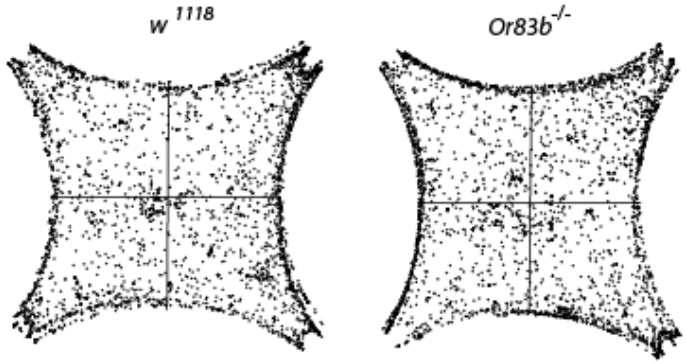



**Figure 8. Density plots of 20 *w*$^{1118}$ and *Or83b*$^{-/-}$ flies in an odor-free arena.**

### 3.4 Visualizing glomerular activity

We next determined which glomeruli are activated by vinegar. We used the genetically encoded calcium sensor G-CaMP to monitor activity in the antennal lobe using two-photon microscopy [4]. We imaged flies bearing the *GH146-Gal4* and *UAS-GCaMP* transgenes, which have G-CaMP expression in 83 out of 150 projection neurons [18-20]. Projection neurons (PNs) are the output neurons of the antennal lobe; thus their responses to odorants contain the information that is important for the behavioral response

We found that, at 3 ppm (the concentration used for the behavioral assay) vinegar elicited a response in six glomeruli out of 34 labeled by *GH146-Gal4*. In the most posterior plane of the antennal lobe, three glomeruli – DM1, DM4, and DP1M – responded quite robustly (Fig. 9b). On a more anterior plane, three more glomeruli – DM2, VA2, and VM2 – also responded to varying degrees (Fig. 9d). Thus, at this behaviorally relevant concentration, vinegar excites six glomeruli. Although vinegar is a complex stimulus with many volatile components, previous studies have shown that several natural stimuli also elicit a surprisingly sparse response in the rodent olfactory bulb [21].

We also imaged ORNs in flies bearing *Or83b-Gal4* and *UAS-GCaMP,* and found that the PN response pattern is similar to the response of the ORNs (Fig. 10), a result that is consistent with previous studies [3, 4]. Although excitatory interglomerular connections do exist [22], recent studies have found that ORN input is the main determinant of PN output [23, 24].

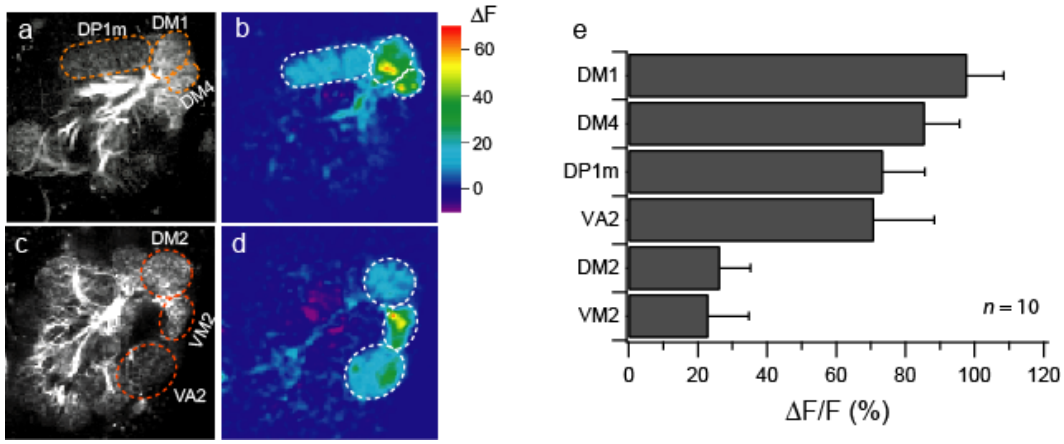



**Figure 9. Six GH-146 expressing glomeruli are activated. a, b,** Pre-stimulation images showing glomerular structure. **c, d,** Responses to 3 ppm vinegar in flies bearing the *GH146-Gal4* and *UAS-GCaMP* transgenes. **e,** Quantification of ΔF/F for all six glomeruli over ten flies. Error bars indicate s.e.m.

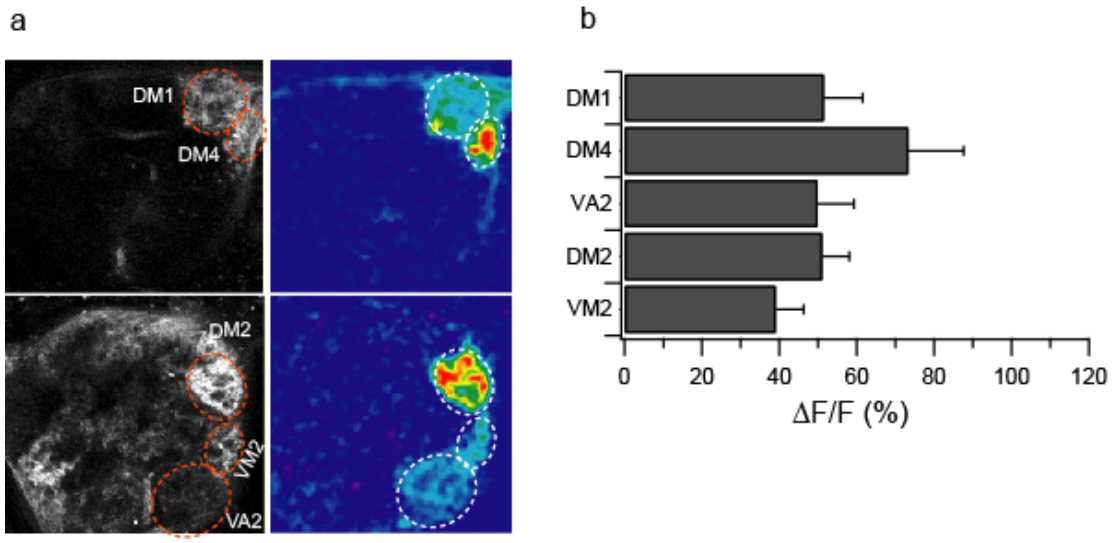



**Figure 10. Vinegar activates a similar pattern of glomeruli when G-CaMP is expressed in ORNs or PNs. a,** Response to 3 ppm vinegar in a fly bearing the *Or83b-Gal4* and *UAS-GCaMP* transgenes. Note that the DP1m glomerulus is not labeled by the *Or83b-Gal4* line. **b,** Average responses in ORN axons from four samples.

### 3.5 Two glomeruli relevant for attraction

In order to determine what role each activated glomerulus plays in mediating the attraction to vinegar, we silenced each ORN channel in turn, and asked how that affects the attraction behavior. Recently, a nearly complete map of ORN to glomerulus targeting was generated [25, 26], so we were able to match five of the six activated glomeruli with their corresponding ORs (the receptor for DP1m remains unknown). *shibire*$^{ts}$ is a temperature sensitive mutant dynamin, which reversibly prevents neurotransmitter release at the non-permissive temperature (32° C) by blocking endocytosis [27]. By generating flies bearing the *UAS-shi*$^{ts}$ transgene and selective Or-Gal4 drivers, we should be able to silence five of the six glomeruli. Indeed, silencing individual ORN types resulted in a dramatic reduction in the activity of their cognate PNs, without affecting the non-cognate PN response (Fig. 11).

We found that when the Or42b neurons, which innervate the DM1 glomerulus, were silenced, the attraction to vinegar was virtually eliminated (Fig. 12b, g). At the non-permissive temperature, the performance index for these flies was -4%, compared to 69% at the permissive temperature. To independently confirm this result, we have measured attraction behavior in an *Or42b* mutant [28] and found a similar attraction deficit (PI = -18 ± 14%, n=18). Silencing the Or92a neurons, which innervate the VA2 glomerulus, also had a marked effect on the behavior, with the performance index declining to 50% at 32° C (Fig. 12c, g). Flies with silenced DM4 and VM2 glomeruli showed normal attraction, as did all the genetic background controls (Fig. 12a, e, f, Fig. 13). The deficits we observed when DM1 or VA2 were silenced suggest that these receptor neuron channels are required for the innate attraction behavior, and could function as labeled

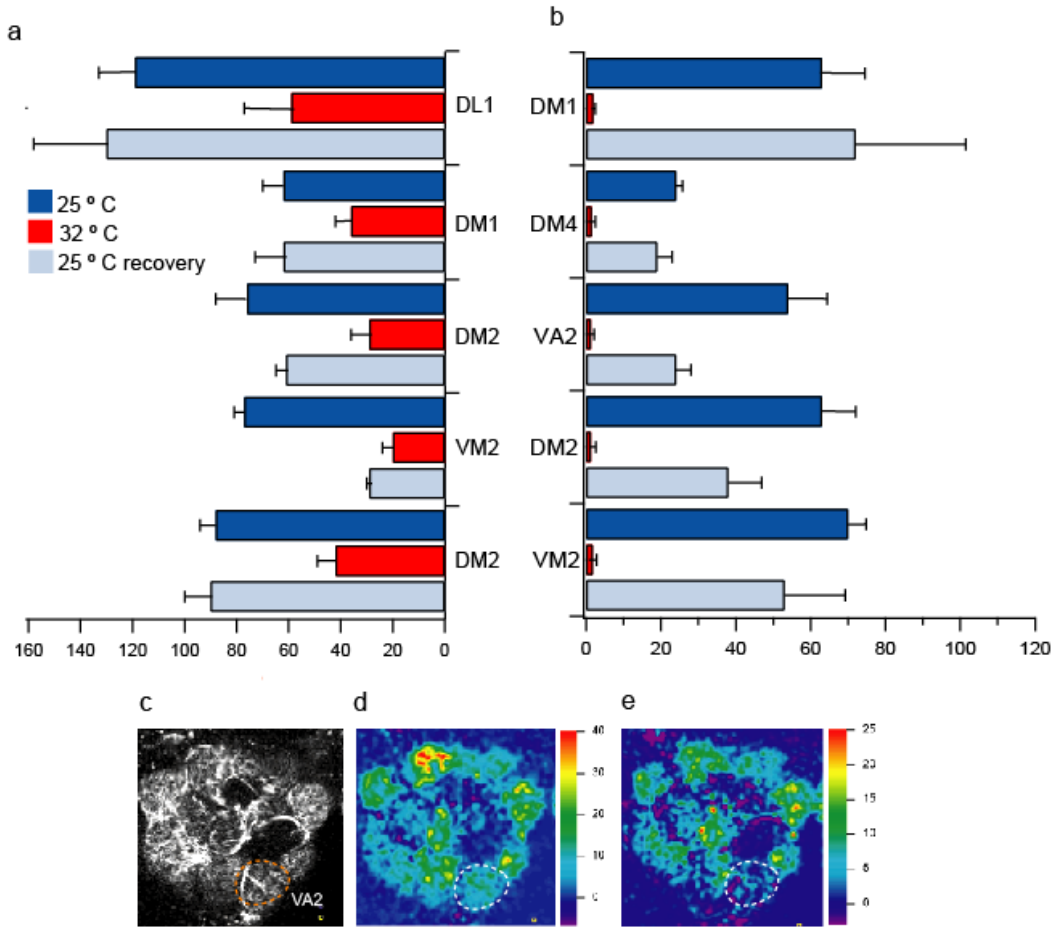



**Figure 11. Shibire$^{ts}$ blocks PN activation at 32° C when expressed in ORNs.** PN responses to electrical stimulation of the antennal nerve were measured in flies with one type of ORNs silenced. **a,** Responses to electrical stimulation in non-silenced glomeruli, from the same preparations as in (**b**), n=3. For example, DL1 in (**a**) was measured from the same preparation as DM1 in (**b**)**. b,** Responses to electrical stimulation in glomeruli where the corresponding ORNs express *shibire$^{ts}$*, n=3. **c.** Pre-stimulation image. **d.** Permissive temperature response in a fly in which the Or92a ORNs are silenced. **e.** Response of the same fly at the non-permissive temperature. Flies contained the following transgenes: *GH146-LexAGAD*, *LexAop-GCaMP-IRES-GCaMP*, *OrX-Gal4*, *UAS-shibire$^{ts}$*. In general, GCaMP fluorescence decreased at 32° C, but for glomeruli where the ORNs were silenced, the response was virtually abolished. The olfactory nerve was stimulated with 16 pulses of 10 V at 1 ms duration and 100 Hz.

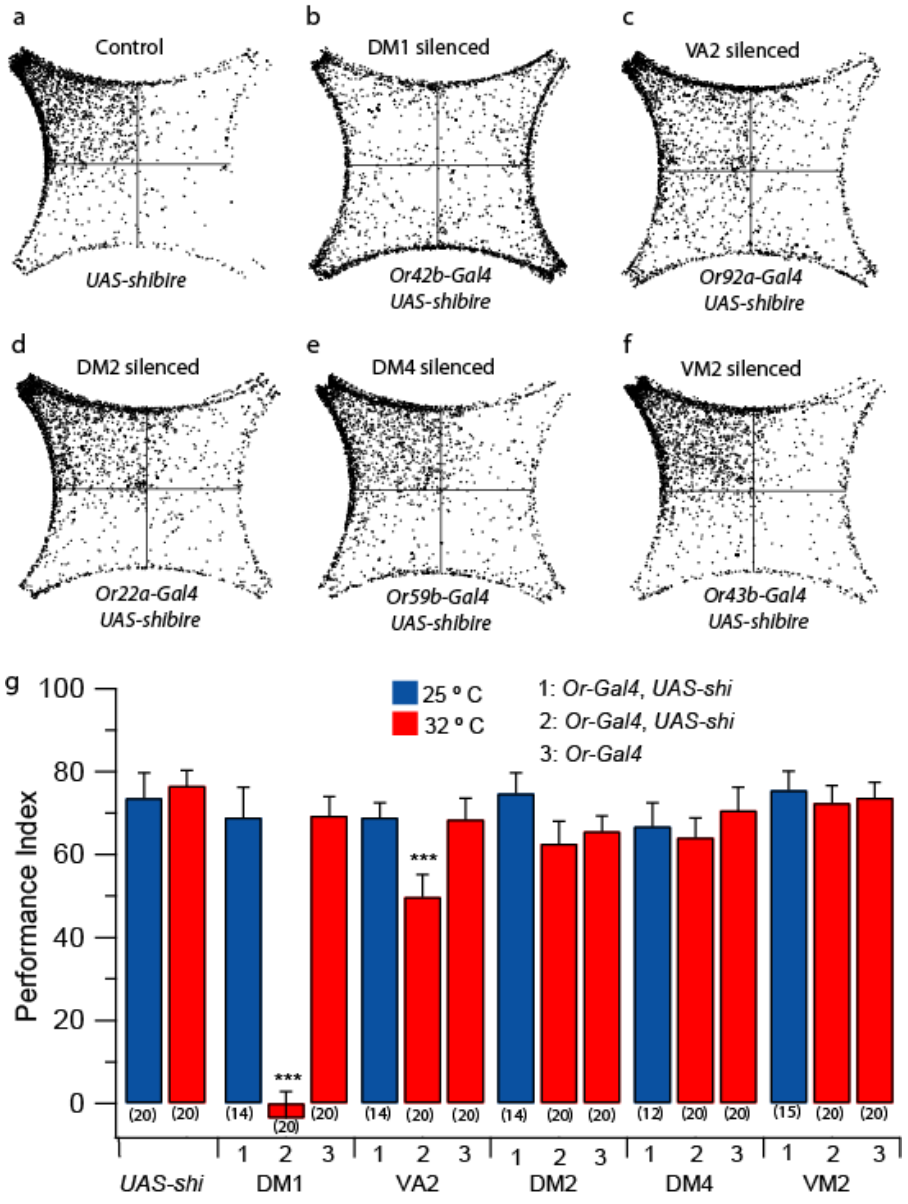



**Figure 12. Silencing DM1 or VA2 reduces attraction to 3 ppm vinegar. a-f,** Density plots composed of 20 flies each. **g,** Performance indices for flies bearing the *OrX-Gal4* and *UAS-shi*$^{ts}$ transgenes at permissive and nonpermissive temperatures. ANOVA followed by Tukey's test was performed on PI values from flies of the experimental group at the permissive and nonpermissive temperatures, and the corresponding genetic background controls at the nonpermissive temperature. ***, $P < 0.001$; and **, $P < 0.01$. Error bars indicate s.e.m.

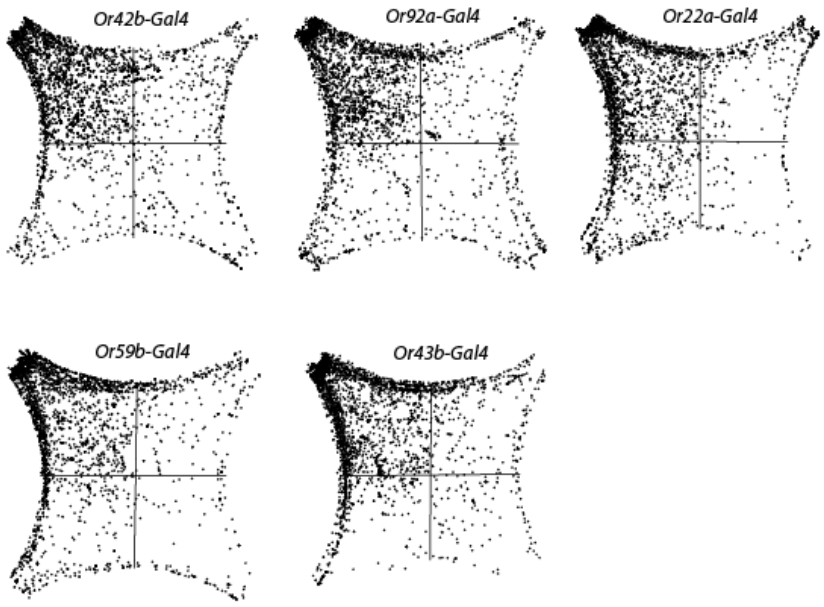



**Figure 13. Or-Gal4 flies exhibit normal attraction.** Density plots of 20 flies each for the five Gal4 lines used in figure 11.

lines for attraction. However, a model in which DM1 and VA2 are necessary for attraction in conjunction with other ORNs would also be consistent with these data.

We next asked whether individual receptor neuron channels could elicit attraction when activated alone. Since *Or83b* mutant flies lack a vital component of the olfactory signaling pathway and are blind to vinegar, we reasoned that by restoring Or83b expression in specific ORNs, we could force vinegar to selectively activate a single Or83b-expressing glomerulus. Thus, we can determine what type of behavioral output each glomerulus would produce. We used *Or-Gal4* lines to drive expression of a *UAS-Or83b* transgene in *Or83b* mutant flies. Calcium imaging experiments confirmed that the rescue flies exhibit normal olfactory responses in the corresponding ORNs (Fig. 14). Remarkably, when the receptor neurons for either DM1 or VA2 were rescued, attraction was restored to normal levels (Fig. 15b, c, g). These results indicate that it is activity in DM1 or VA2, and not the pattern of the six glomeruli, which is read out by higher brain centers to signal the attractiveness of the odor. The finding that VA2 activity is sufficient for attraction may appear inconsistent with the fact that DM1 silenced flies show no attraction to vinegar. However, VA2 may be more robustly activated in the rescue flies, because in the silencing experiments, activation of several remaining ORN channels could result in inhibition of VA2. Indeed, a recent study has shown that adding receptor channel inputs increases lateral inhibition, leading to a reduction in the PN response [29].

### 3.6 Discussion

Two recent studies of olfactory behavior in *Drosophila* larvae have addressed the question of how receptor activation leads to behavioral output. Although there are significant numerical differences in cell numbers between larval and adult olfactory systems, the basic organization is similar. It has been discovered that the responses of five ORNs to a panel of odorants can be used to generate a model which accounts for the 81% of the variation in olfactory behavior [30]. It is somewhat surprising that this linear model, in which ORNs act independently rather than in combination, can predict innate behavior with high accuracy. Selective activation of these ORNs should generate robust innate attraction or aversion. In fact, the Or42a ORN, one of the five critical ORNs, has been shown to be sufficient for attraction behavior [31]. It will be important to determine whether the other four ORNs are sufficient to evoke behavior. In this study, we used calcium imaging to identify the seven glomeruli activated by vinegar, which guided our molecular manipulations to link glomerular response with behavior in adult flies. We found that two of the seven glomeruli mediate attraction and one mediates aversion, providing conclusive evidence that within the pattern of glomeruli activated by a given odor, some make a greater contribution than others to the behavioral output.

### 3.7 Methods

**Behavioral assay.** An existing behavioral paradigm was modified to measure the response of single flies to odors [10]. As previously described, the four field olfactometer consisted of a four-pointed star-shaped arena 30 cm across diagonally and 1 cm deep, covered by a glass plate. Air flow was maintained by vacuum suction such that air entered each quadrant at a rate of 200 mL/min, after passing through a

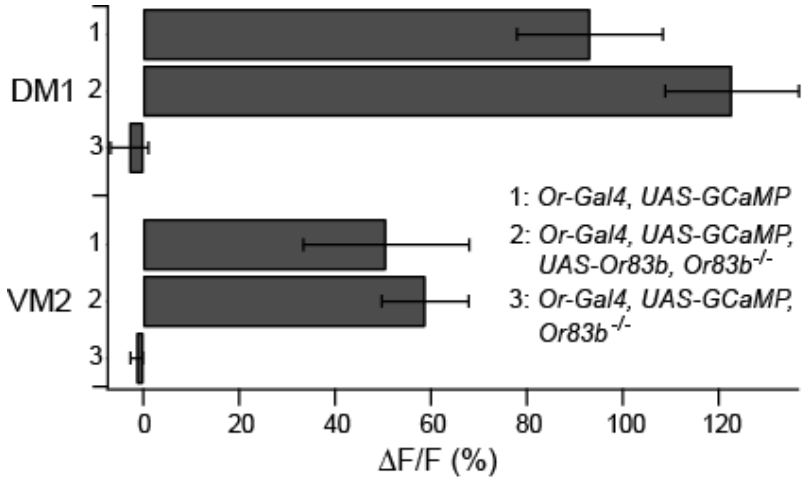



**Figure 14. Expression of Or83b in individual ORNs in *Or83b* mutant flies restores the odorant response to wild-type levels.** Responses to 3 ppm vinegar in flies bearing the indicated transgenes. n≥3.

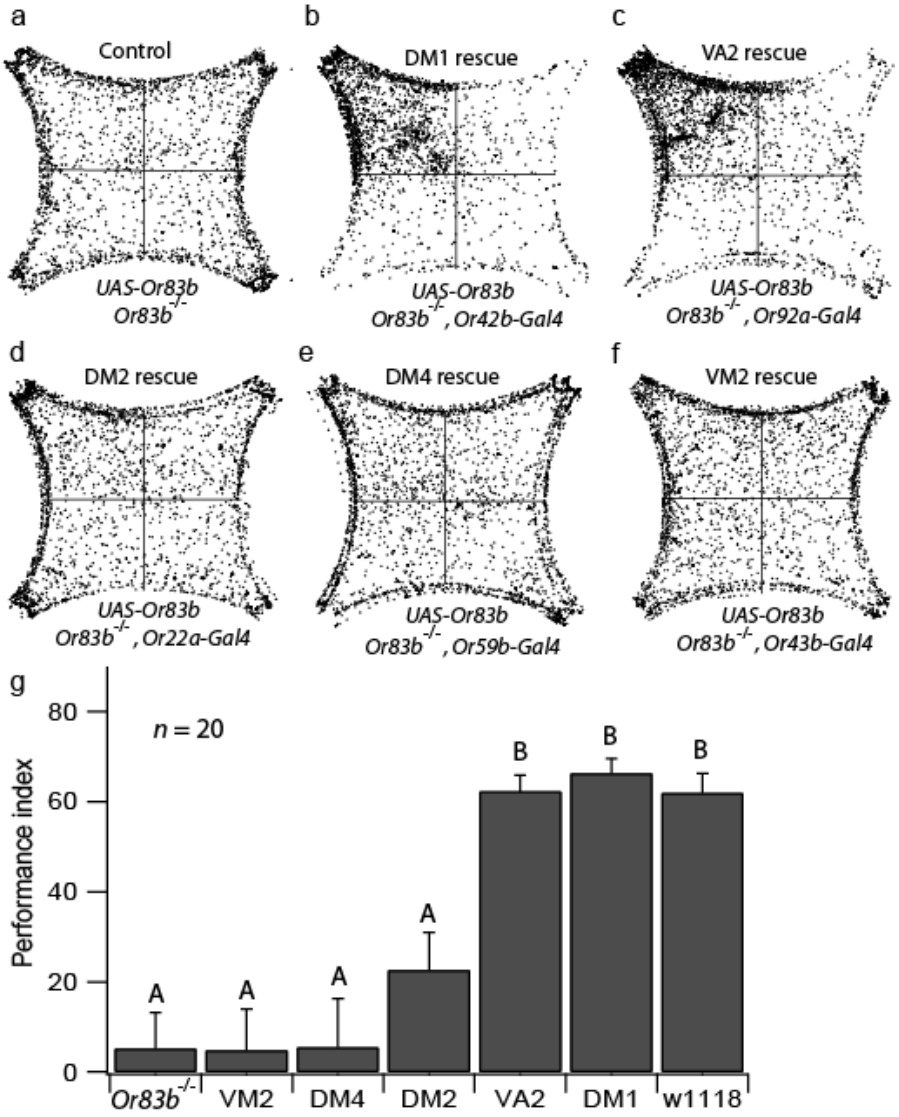



**Figure 15. Restoring *Or83b* in DM1 and VA2 ORNs restores attraction to control levels. a-f,** Density plots of 20 flies responding to 3 ppm vinegar. **g,** Performance indices of flies in which *Or83b* is selectively restored in individual ORN types. Comparisons between groups were made using ANOVA followed by Tukey's test. Significant differences ($P < 0.05$) are denoted by letters. Error bars indicate s.e.m.

100 mL bottle. Only female flies were used, and at the time of the assay the flies were four days old and had been starved for 50 hours in a vial with a wet kimwipe. After a single fly was introduced into the chamber, its speed was measured for 100 seconds, and only flies with an average speed between 0.5 and 1.0 cm/second were used. At the start of the assay, one of the empty 100 mL bottles was replaced with an odor-containing bottle. The fly's location was measured once per second using a Logitech quickcam and Labview software (National Instruments). The chamber was illuminated by a panel of LEDs (660 nm). Light reflected from the glass plate was eliminated by polarizing optics. The performance index is defined as $(2p^{1/2}-1) \times 100\%$, were p is the fraction of time the fly spends in the odor quadrant during the period between 50 and 250 seconds after odor application. Thus, if the fly is in the odor quadrant for the entire time window, p=1 and the performance index is 100%, whereas if the fly avoids the odor quadrant entirely, p=0 and the performance index will be -100%. Except for the *shibire* $^{ts}$ nonpermissive temperature experiments (which were performed at 32° C), all behavioral experiments were performed at 25° C and 70% humidity. Data were analyzed using Igor Pro (Wavemetrics) and a custom macro. The Jarqe-Bera test was used to verify that the data were normally distributed. Density plots show data collected between 50 and 250 seconds for 20 flies. Each dot indicates one fly spending one second at that location. Odor application was alternated among the four quadrants, and the density plots were created by rotating the positional data so that the odor quadrant becomes the upper left quadrant.

**Odor stimuli.** Odor concentration was measured using a photoionization detector (Rae Systems, MiniRAE 2000) and an air flow of 200 mL/min through a 100 mL bottle containing the odorant. As the conversion factor to determine the exact concentration of

cider vinegar volatiles is unknown, we express the concentration in isobutylene equivalents. The 3 ppm concentration of vinegar corresponds to 40 uL of a 1:2 dilution of apple cider vinegar with water on filter paper. The odor source was replenished for each experiment. Odor concentrations stayed constant over the time course of an experiment.

**G-CaMP imaging experiments.** Calcium imaging was performed as described [4, 32], except that the air flow rate was 200 mL/min. Odorants were administered from 100 mL bottles as described above, and stimuli were given for 2 seconds.

**Transgenic Flies.** The following fly stocks were used: *Or42b-Gal4, Or43b-Gal4, Or92a-Gal4, Or22a-Gal4*, and *Or92a-Gal4* [25]; *Or59b-Gal4* [26]; *UAS-Or83b*, *Or83b* targeted deletion ($Or83b^2$) [16]; $UAS\text{-}shibire^{ts}$ [27]; *UAS-GCaMP* [4]; *GH146-Gal4* [20]; *GH146-LexAGAD* [33]; *LexAop-GCaMP-IRES-GCaMP* [32].

**Behavioral Analysis.** The following Igor procedure was used to calculate performance indices and generate density maps:

```
#pragma rtGlobals=1                    // Use modern global access method.

Macro CalculatePI()
        DeclPIVar()
        Main_Panel()
End

Function DeclPIVar()
        Variable/G frame = 600
        Make/N=600/D/O XX
        Make/N=600/D/O YY
```

```
	Make/N=600/D/O XXo

	Make/N=600/D/O YYo

	Make/N=600/D/O Q1

	Make/N=600/D/O Q2

	Make/N=600/D/O Q3

	Make/N=600/D/O Q4

	Make/N=600/D/O Performance

	Make/N=600/D/O Distance

	Make/N=1/D/O XXB, YYB

	Variable/G n_fly = 1

	Variable/G m =0

	Variable/G n=0

	Variable/G j=0

	Variable/G dim=0

End


//**************     PANELS **************

Window Main_Panel() : Panel

	PauseUpdate; Silent 1		| building window...

	NewPanel /W=(7,47,207,607)

	ModifyPanel cbRGB=(2,39321,1)

	Button
BUTTON_READ_Text,pos={40,20},size={120,40},proc=ButtonReadTextFile,title="Read Text File"

	Button BUT_Location,pos={40,80},size={120,40},proc=ButtonFlyLocation,title="Fly Location"

	Button BUT_PI,pos={40,140},size={120,40},proc=ButtonFlyPI,title="Performance"

	Button BUT_Q1,pos={10,200},size={80,40},proc=ButtonQ1,title="Odor@Q1"

	Button BUT_Q2,pos={110,200},size={80,40},proc=ButtonQ2,title="Odor@Q2"

	Button BUT_Q3,pos={110,260},size={80,40},proc=ButtonQ3,title="Odor@Q3"
```

```
	Button BUT_Q4,pos={10,260},size={80,40},proc=ButtonQ4,title="Odor@Q4"
	Button BUT_StartD,pos={40,320},fColor=(65280,43520,0),
size={120,40},proc=ButtonStartDensity,title="Start Density/Erase"
	Button
BUT_DisplayD,pos={40,380},fColor=(65280,43520,0),size={120,40},proc=ButtonDisplayDensity,title="
Display Density"
	Button BUT_CalculateD,pos={40,440},fColor=(65280,43520,0),
size={120,40},proc=ButtonCalculateDensity,title="Calcualte Density"
	Button BUT_Density, pos={40, 500}, fColor=(65280,43520,0),
size={120,40},proc=ButtonDensityImage,title="Density Image"
	ValDisplay valdisp0, value=n_fly, pos={145,450}, disable=2, fsize=14, bodywidth=30
EndMacro

Function ButtonReadTextFile(ctrlName) : ButtonControl
	String ctrlName
	NVAR frame
	Wave XX,YY,Q1,Q2,Q3,Q4, XXo, YYo, Performance
	LoadWave/A/O/G/B="N=XX; N=YY; N=Q1; N=Q2; N=Q3; N=Q4;N=XXo; N=YYo;"
	frame = numpnts(XX)
	Variable i = frame-1
	do
		if (XX[i] < 1)
			XX[i] = XX[i+1]
			YY[i] = YY[i+1]                                    //Some frames fail to detect the
fly position. In these frames, XX =0
			Q1[i] = Q1[i+1]                                    //Use data from
neighboring frames.
			Q2[i] = Q2[i+1]
```

```
                Q3[i] = Q3[i+1]
                Q4[i] = Q4[i+1]
            endif
            i = i - 1
        while (i >= 0)

        XX = XX- XXo                        //Make center (0,0)
        YY = YY - YYo
        Duplicate/R=(0,0)/O XX XXB          // define beginning point
        Duplicate/R=(0,0)/O YY YYB
        Duplicate/O XX, Performance
        Duplicate/O XX, Distance
        Distance [ ] = sqrt((XX[p]-XX[p-1])^2+(YY[p]-YY[p-1])^2)
        Q2 = Q2/2
        Q3 = Q3/3
        Q4 = Q4/4
        i = frame -1
        do
            if (Distance[i] > 10*mean(Distance))          //Eliminate tracking errors,
//distance between consecutive frames larger than 10*average is not possible
                DeletePoints i, 1, XX, YY, Distance, Q1, Q2, Q3, Q4, Performance
            endif

            Distance [ ] = sqrt((XX[p]-XX[p-1])^2+(YY[p]-YY[p-1])^2)
            i = i -1
        while (i >=0)
        i = frame -1
        do
```

```
                if (Distance[i] > 10*mean(Distance))                    //Eliminate tracking errors,
//distance between consecutive frames larger than 10*average is not possible
                        DeletePoints i, 1, XX, YY, Distance, Q1, Q2, Q3, Q4, Performance
                endif

                Distance [ ] = sqrt((XX[p]-XX[p-1])^2+(YY[p]-YY[p-1])^2)
                i = i -1
        while (i >=0)
        i = frame -1
        do
                if (Distance[i] > 10*mean(Distance))                    //Eliminate tracking errors,
//distance between consecutive frames larger than 10*average is not possible
                        DeletePoints i, 1, XX, YY, Distance, Q1, Q2, Q3, Q4, Performance
                endif
                Distance [ ] = sqrt((XX[p]-XX[p-1])^2+(YY[p]-YY[p-1])^2)
                i = i -1
        while (i >=0)
        if (0.95*frame > numpnts(XX))
                abort "Tracking error! Discard sample"                                  // //Discard
sample when more than 5% frames have problem
        endif
End

Function ButtonFlyLocation(ctrlName) : ButtonControl
        String ctrlName
        PauseUpdate; Silent 1
        Display/W=(207,47,807,647) YY vs XX
        ModifyGraph width=288,height=288
```

```
        ModifyGraph mode=0
        SetAxis/A/R left
        AppendToGraph YYB vs XXB
        ModifyGraph mode(YYB)=3,marker(YYB)=19,rgb(YYB)=(0,0,0)
        SetDrawEnv xcoord= bottom;DelayUpdate
        DrawLine 0,1,0,0
        SetDrawEnv ycoord= left;DelayUpdate
        DrawLine 0,0,1,0
        SetAxis/R left 350,-350;DelayUpdate
        SetAxis bottom -350, 350
End

Macro ButtonQ1(ctrlName) : ButtonControl
        String ctrlName
        Performance = Q1
        Print "Attraction Index = ", 200*SQRT(sum(Performance, 50, 249)/200)-100 //          Average
between f50 and f250
End

Macro ButtonQ2(ctrlName) : ButtonControl
        String ctrlName
        Performance = Q2
        Duplicate/O XX, XXi
        Duplicate/O YY, YYi
        YY = -XXi                       //90 degree rotation
        XX = YYi
        Print "Attraction Index = ", 200*SQRT(sum(Performance, 50, 249)/200)-100
EndMacro
```

```
Macro ButtonQ3(ctrlName) : ButtonControl
	String ctrlName
	Performance = Q3
	Duplicate/O XX, XXi
	Duplicate/O YY, YYi
	YY = -XXi 				//180 degree rotation
	XX = -YYi
	Print "Attraction Index = ", 200*SQRT(sum(Performance, 50, 249)/200)-100
EndMacro

Macro ButtonQ4(ctrlName) : ButtonControl
	String ctrlName
	Performance = Q4
	Duplicate/O XX, XXi
	Duplicate/O YY, YYi
	YY = XXi 				//270 degree rotation
	XX = -YYi
	Print "Attraction Index = ", 200*SQRT(sum(Performance, 50, 249)/200)-100
EndMacro

Macro ButtonFlyPI(ctrlName): ButtonControl
	String ctrlName
	Display Distance
	SetAxis left 0,250
	AppendToGraph/L=L4 Q1
	AppendToGraph/L=L3 Q2
	AppendToGraph/L=L2 Q3
```

```
	AppendToGraph/L=L1 Q4
	ModifyGraph axisEnab(left)={0,0.4},axisEnab(L1)={0.42,0.55};DelayUpdate
	ModifyGraph axisEnab(L2)={0.57,0.7},axisEnab(L3)={0.72,0.85},axisEnab(L4)={0.87,1}
	ModifyGraph noLabel(left)=2,noLabel(L1)=2,noLabel(L2)=2,noLabel(L3)=2;DelayUpdate
	ModifyGraph noLabel(L4)=2,axThick(left)=0,axThick(L1)=0,axThick(L2)=0;DelayUpdate
	ModifyGraph axThick(L3)=0,axThick(L4)=0
	ModifyGraph rgb(Q1)=(0,0,0),rgb(Q2)=(0,0,0),rgb(Q3)=(0,0,0),rgb(Q4)=(0,0,0)
	SetDrawEnv ycoord= left,linefgc= (0,0,65280),dash= 3;DelayUpdate
	DrawLine 0,0,1,0
EndMacro

Function ButtonStartDensity(ctrlName):    ButtonControl
String ctrlName
NVAR n_fly
Duplicate/O/R=(50,249) XX, XXX                    //Use only frame 50 to 249.
Duplicate/O/R=(50,249) YY, YYY
n_fly = 1
End

Function ButtonCalculateDensity(ctrlName): ButtonControl
String ctrlName
NVAR n_fly
Wave XXX, YYY
Duplicate/O/R=(50,249) XX, XXN                    //Use only 200 frames for density //calculation
Duplicate/O/R=(50,249) YY, YYN
Concatenate/O/NP/KILL {XXX,XXN}, XXX
Concatenate/O/NP/KILL {YYY,YYN}, YYY
n_fly = n_fly + 1
```

```
End


Function ButtonDensityImage(ctrlName): ButtonControl
String ctrlName
Wave XXX, YYY
NVAR m,n,j,dim
Make/N=(900,900)/B/O Density
Make/N=(90,90)/B/O Density_Bin
Make/N=(10,10)/B/O Block
Density = 0
Density_Bin = 0
dim = numpnts(XXX)
XXX = XXX+450
YYY = YYY+450
j = 0                        //Calculate Density
do
        m = XXX[ j ]
        n = YYY[ j ]
        Density[m][n] = Density[m][n] + 1
        j += 1
while (j < dim)
m = 0
n = 0
do                                  //10x10 binning
        n = 0
        do
        Block = Density [10*m+p] [10*n+q]
        Density_Bin[m][n] = sum(Block)
```

```
        n +=1
        while (n < 90)
m +=1
while (m <90)
End

Function ButtonDisplayDensity(CtrlName): ButtonControl
String ctrlName
SetAxis/A/R left
        PauseUpdate; Silent 1
        Display/W=(207,47,807,647) YYY vs XXX
        ModifyGraph width=288,height=288
        ModifyGraph mode=0
        SetAxis/A/R left
        SetDrawEnv xcoord= bottom;DelayUpdate
        DrawLine 0,1,0,0
        SetDrawEnv ycoord= left;DelayUpdate
        DrawLine 0,0,1,0
        ModifyGraph mode=3,marker=8,msize=1
End
```

This chapter, with chapter 4, was published in Nature in 2009 under the title "Select *Drosophila* glomeruli mediate innate olfactory attraction and aversion". The dissertation author was the primary author of this paper, with Jing Wang as co-author.

**References**


1. de Bruyne, M., K. Foster, and J.R. Carlson, *Odor coding in the Drosophila antenna.* Neuron, 2001. **30**(2): p. 537-52.

2. Hallem, E.A., M.G. Ho, and J.R. Carlson, *The molecular basis of odor coding in the Drosophila antenna.* Cell, 2004. **117**(7): p. 965-79.

3. Ng, M., et al., *Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly.* Neuron, 2002. **36**(3): p. 463-74.

4. Wang, J.W., et al., *Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain.* Cell, 2003. **112**(2): p. 271-82.

5. Fiala, A., et al., *Genetically expressed cameleon in Drosophila melanogaster is used to visualize olfactory information in projection neurons.* Curr Biol, 2002. **12**(21): p. 1877-84.

6. Suh, G.S., et al., *A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila.* Nature, 2004. **431**(7010): p. 854-9.

7. Kurtovic, A., A. Widmer, and B.J. Dickson, *A single class of olfactory neurons mediates behavioural responses to a Drosophila sex pheromone.* Nature, 2007. **446**(7135): p. 542-6.

8. Clyne, P., et al., *Odorant response of individual sensilla on the Drosophila antenna.* Invert Neurosci, 1997. **3**(2-3): p. 127-35.

9. Ha, T.S. and D.P. Smith, *A pheromone receptor mediates 11-cis-vaccenyl acetate-induced responses in Drosophila.* J Neurosci, 2006. **26**(34): p. 8727-33.

10. Faucher, C., et al., *Behavioral responses of Drosophila to biogenic levels of carbon dioxide depend on life-stage, sex and olfactory context.* J Exp Biol, 2006. **209**(Pt 14): p. 2739-48.

11. Suh, G.S., et al., *Light activation of an innate olfactory avoidance response in Drosophila.* Curr Biol, 2007. **17**(10): p. 905-8.

12. Stensmyr, M.C., et al., *Novel natural ligands for Drosophila olfactory receptor neurones.* J Exp Biol, 2003. **206**(Pt 4): p. 715-24.

13. Zhu, J., K.C. Park, and T.C. Baker, *Identification of odors from overripe mango that attract vinegar flies, Drosophila melanogaster.* J Chem Ecol, 2003. **29**(4): p. 899-909.

14. Vosshall, L.B., A.M. Wong, and R. Axel, *An olfactory sensory map in the fly brain.* Cell, 2000. **102**(2): p. 147-59.

15. Sato, K., et al., *Insect olfactory receptors are heteromeric ligand-gated ion channels.* Nature, 2008. **452**(7190): p. 1002-U9.

16. Larsson, M.C., et al., *Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction.* Neuron, 2004. **43**(5): p. 703-14.

17. Wicher, D., et al., *Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels.* Nature, 2008. **452**(7190): p. 1007-U10.

18. Wong, A.M., J.W. Wang, and R. Axel, *Spatial representation of the glomerular map in the Drosophila protocerebrum.* Cell, 2002. **109**(2): p. 229-41.

19. Vosshall, L.B. and R.F. Stocker, *Molecular architecture of smell and taste in Drosophila.* Annu Rev Neurosci, 2007. **30**: p. 505-33.

20. Stocker, R.F., et al., *Neuroblast ablation in Drosophila P[GAL4] lines reveals origins of olfactory interneurons.* J Neurobiol, 1997. **32**(5): p. 443-56.

21. Lin, D.Y., S.D. Shea, and L.C. Katz, *Representation of natural stimuli in the rodent main olfactory bulb.* Neuron, 2006. **50**(6): p. 937-49.

22. Shang, Y., et al., *Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe.* Cell, 2007. **128**(3): p. 601-12.

23. Olsen, S.R., V. Bhandawat, and R.I. Wilson, *Excitatory interactions between olfactory processing channels in the Drosophila antennal lobe.* Neuron, 2007. **54**(1): p. 89-103.

24. Root, C.M., et al., *Propagation of olfactory information in Drosophila.* Proc Natl Acad Sci U S A, 2007. **104**(28): p. 11826-31.

25. Fishilevich, E. and L.B. Vosshall, *Genetic and functional subdivision of the Drosophila antennal lobe.* Curr Biol, 2005. **15**(17): p. 1548-53.

26. Couto, A., M. Alenius, and B.J. Dickson, *Molecular, anatomical, and functional organization of the Drosophila olfactory system.* Curr Biol, 2005. **15**(17): p. 1535-47.

27. Kitamoto, T., *Conditional modification of behavior in Drosophila by targeted expression of a temperature-sensitive shibire allele in defined neurons.* J Neurobiol, 2001. **47**(2): p. 81-92.

28. Bellen, H.J., et al., *The BDGP gene disruption project: single transposon insertions associated with 40% of Drosophila genes.* Genetics, 2004. **167**(2): p. 761-81.

29. Asahina, K., et al., *A circuit supporting concentration-invariant odor perception in Drosophila.* J Biol, 2009. **8**(1): p. 9.

30. Kreher, S.A., et al., *Translation of sensory input into behavioral output via an olfactory system.* Neuron, 2008. **59**(1): p. 110-24.

31. Fishilevich, E., et al., *Chemotaxis behavior mediated by single larval olfactory neurons in Drosophila.* Curr Biol, 2005. **15**(23): p. 2086-96.

32. Root, C.M., et al., *A presynaptic gain control mechanism fine-tunes olfactory behavior.* Neuron, 2008. **59**(2): p. 311-21.

33. Lai, S.L., et al., *Clonal analysis of Drosophila antennal lobe neurons: diverse neuronal architectures in the lateral neuroblast lineage.* Development, 2008. **135**(17): p. 2883-93.

# Chapter 4

## 4.1 Abstract

As the concentration of an odorant is increased, additional receptors are often activated, and the behavioral output may change. We increased the concentration of apple cider vinegar and found that it became markedly less attractive. Futhermore, an additional glomerulus, DM5, was activated. Silencing and rescue experiments implicated DM5 in the behavioral switch. Activation of DM5 and DM1 with a monomolecular odorant, ethyl butyrate, proved to be sufficient for attraction and repulsion, respectively. Sensitization of the DM5 glomerulus through expression of a different OR shifted the behavioral response toward aversion.

## 4.2 Introduction

As odor concentration is increased, odors that are attractive at low concentrations often become less attractive or even repulsive [1]. Increasing the odorant concentration often recruits additional receptor neurons, and thus it has been proposed that the change in behavior is mediated by the addition of these glomeruli to the ensemble of activated glomeruli [2], but this hypothesis has not been tested directly. It is also possible that increased activation of the glomeruli that were active at the low concentration could mediate the change in behavioral output [3]. Alternatively, the new glomeruli could independently induce aversion.

## 4.3 A concentration dependent behavioral switch

We first measured the olfactory behavior over a range of vinegar concentrations. As we increased the concentration, we observed a slight increase in attractiveness at 12

ppm (Fig. 16a) but then a striking decrease in attractiveness at 32 ppm, with the performance index dropping to 9% (Fig. 16a). We wondered whether the change could be due to recruitment of additional glomeruli, and used calcium imaging to determine the difference in the pattern of glomeruli activated in response to 12 and 32 ppm vinegar. We observed that the DM5 glomerulus, which showed no response at 12 ppm, was strongly activated by 32 ppm (Fig. 16d bottom row, and e). All other glomeruli that were activated by 12 ppm (DM1, DM4, DP1m, DM2, DM3, VM2, and VA2) showed small to moderate increases in response to 32 ppm vinegar.

We next asked whether DM5 could be responsible for the decrease in attraction to vinegar observed at 32 ppm. Therefore, we silenced the DM5 glomerulus by expressing *shibire* $^{ts}$ in its cognate ORNs, which express Or85a. At the nonpermissive temperature, we found that the performance index for 32 ppm vinegar increased to 87% (Fig. 17a, c). In contrast, silencing DM1 resulted in repulsion towards 32 ppm vinegar (Fig. 17c). Thus, the activation of DM5 is responsible for the decrease in attractiveness towards 32 ppm vinegar.

In light of the above result, it is possible that the activation of DM5 alone mediates aversion, or that activation of DM5 together with other specific glomeruli could mediate aversion. To distinguish between these models, we forced the stimulus to activate only DM5 by expressing Or83b in Or85a ORNs in the *Or83b* mutant background. We found that these flies were repulsed by 32 ppm vinegar, whereas the *Or83b* mutant flies showed no preference or aversion to the odorant (Fig. 17b, c). In contrast, when DM1 was selectively activated by expression of *Or83b* in Or42b ORNs, flies were attracted to 32 ppm vinegar (Fig. 17c and Fig. 18). These findings

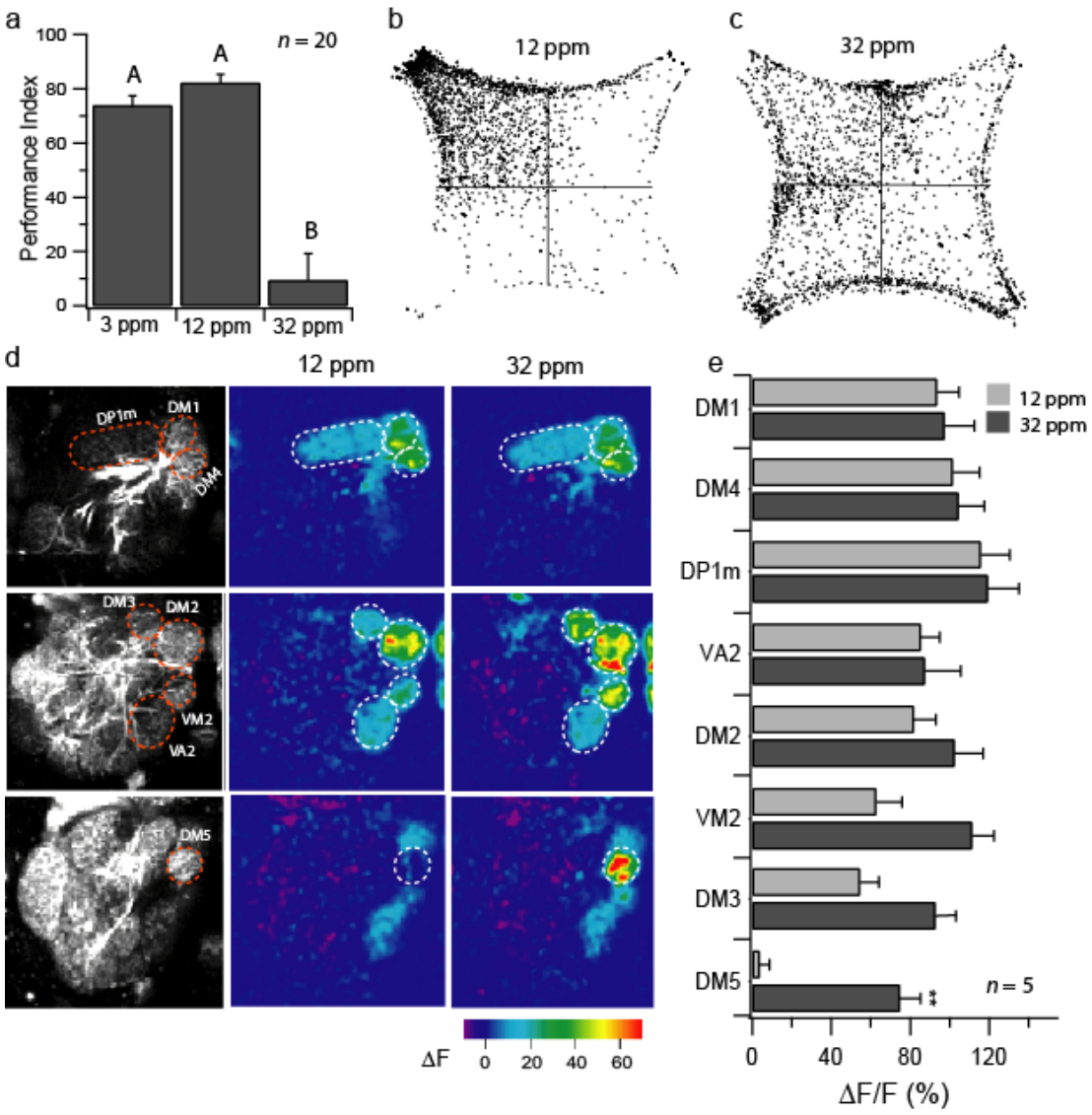



**Figure 16. Vinegar becomes less attractive and activates an additional glomerulus at high concentrations. a,** Performance indices of $w^{1118}$ flies at various concentrations of vinegar. PI values were compared using ANOVA followed by Tukey's test. Significant differences ($P < 0.05$) are denoted by letters. **b,c,** Density plots of $w^{1118}$ behavior in response to 12 ppm and 32 ppm vinegar. **d,** Responses to 12 ppm and 32 ppm vinegar in flies bearing the *GH146-Gal4* and *UAS-GCaMP* transgenes**. e,** Average ΔF/F. ** indicates $P < 0.01$; T-test. Error bars indicate s.e.m.

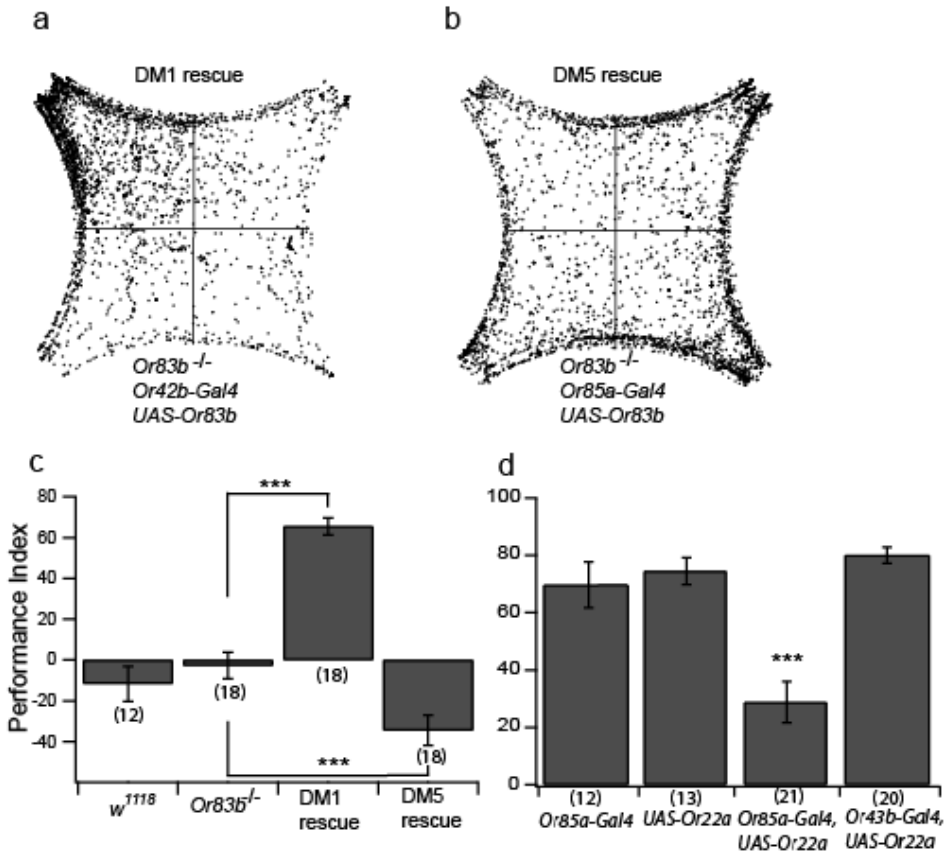



**Figure 17. DM5 mediates the decrease in attraction in response to 32 ppm vinegar. a,** Density plot of twenty flies in which the DM5 ORNs are silenced. **b,** Density plot of 20 DM5 rescue flies. **c,** Behavioral responses to 32 ppm vinegar for flies in which DM5 and DM1 are silenced and selectively rescued. For silencing experiments, we performed the same statistical analysis as for Figure 2. DM5 rescue and DM1 rescue flies were compared to *Or83b*$^{-/-}$ flies by T-test. ***, $P < 0.001$; and **, $P < 0.01$.

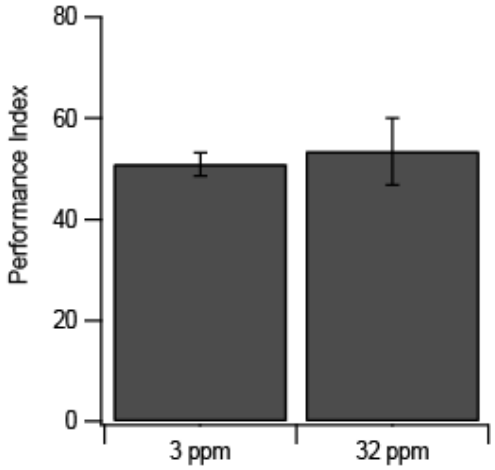



**Figure 18. DM1 rescue flies are attracted to 32 ppm vinegar.** Behavior of *Or83b*$^{-/-}$ flies bearing the *Or42b-Gal4* and *UAS-Or83b* transgenes.

suggest that the higher concentration of vinegar recruits an additional glomerulus that independently mediates aversion. When wild type flies are exposed to 32 ppm vinegar, the activation of an aversive glomerulus may counterbalance the activation of the two attractive glomeruli, resulting in a PI near zero.

### 4.4 Monomolecular odor

If attraction and aversion are mediated by the activation of specific glomeruli, other odors that activate these glomeruli should give the same behavioral output. For example, an odor that excites DM1 should be attractive to flies in which DM1 ORNs are selectively activated, while an odor that selectively excites DM5 should be repulsive. We have identified an odorant, ethyl butyrate, that excites the DM1, DM2, VM2 and DM5 glomeruli (Fig. 19) but has not been detected by gas chromatography in cider vinegar [4]. When we selectively restored function in DM1 ORNs, we found that ethyl butyrate triggered attraction behavior, with a PI of 65%. Conversely, when we selectively restored function in the DM5 ORNs, the result was an aversion to ethyl butyrate, with a PI of -34% (Fig. 20a). These results suggest that the activation of DM1 or DM5 by any odor should be sufficient for attraction and aversion, respectively.

If specific glomeruli are hardwired to generate attraction and aversion behavior, activation of ectopically expressed receptors should give a similar behavioral output. We predict that expression of the *Or22a* receptor in Or85a ORNs, which project to DM5, should make these neurons sensitive to lower concentrations of vinegar, and bias the behavior towards aversion. Indeed, these flies exhibit a dramatic reduction in PI value

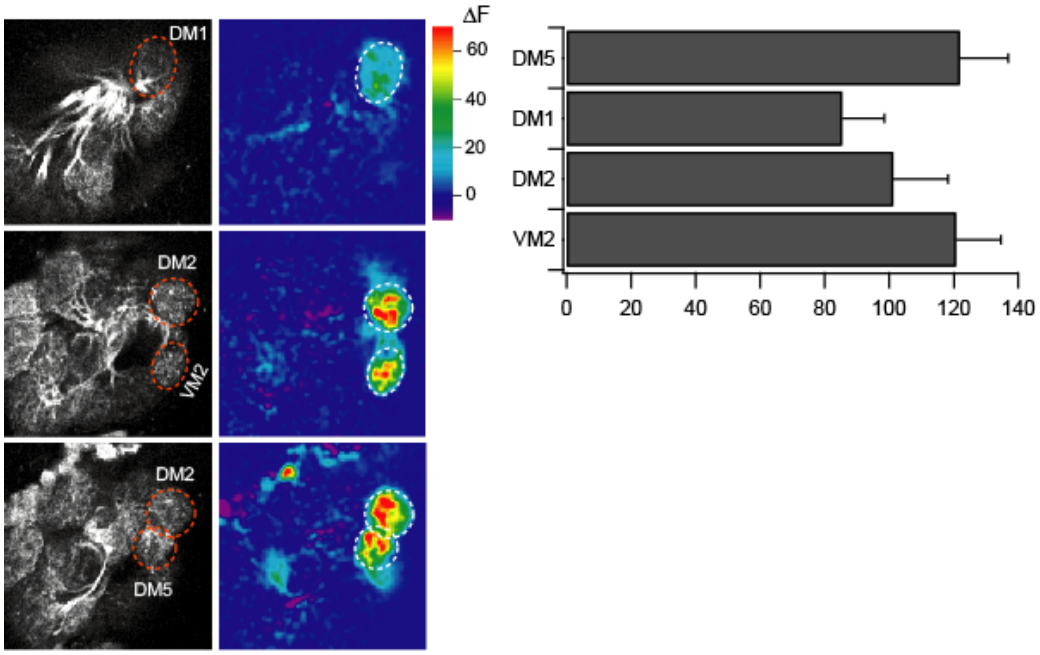



**Figure 19. Ethyl butyrate activates four glomeruli. a,** Response to 7 ppm ethyl butyrate in a fly bearing the *GH146-Gal4* and *UAS-GCaMP* transgenes. **b**, Average response to ethyl butyrate, n=4.

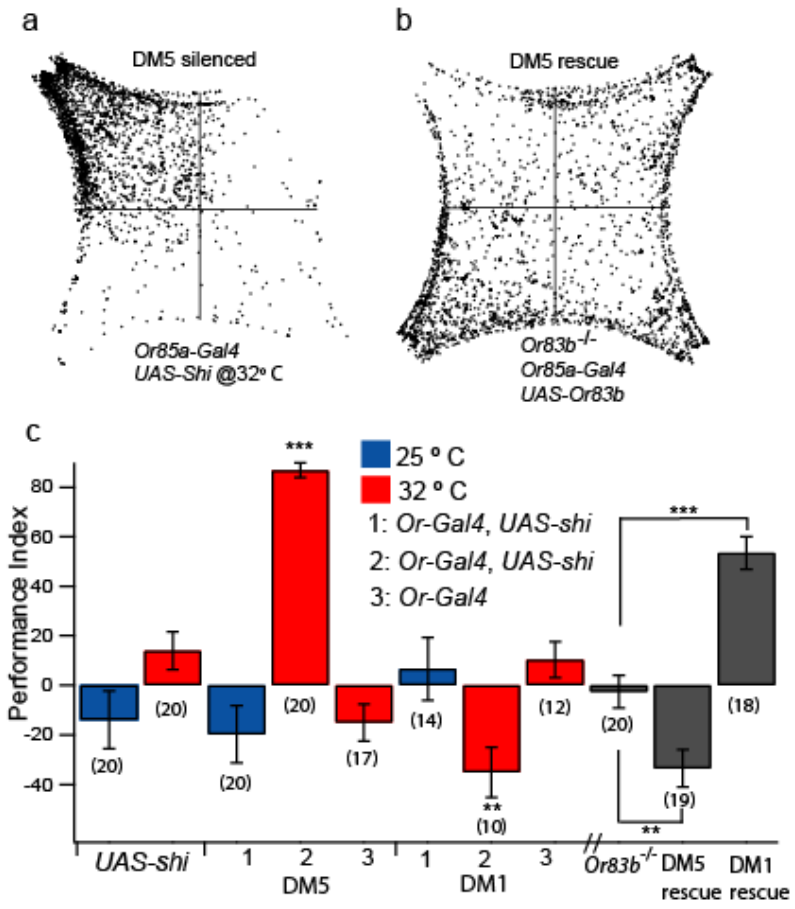



**Figure 20. DM1 and DM5 mediate attraction and aversion in response to ethyl butyrate. a,** Performance indices in response to 7 ppm ethyl butyrate for DM1 and DM5 rescue flies. ***, $P < 0.001$; T-test. **b,** Ectopic expression of Or22a in Or85a ORNs reduced attraction to 12 ppm vinegar. PI values were compared using ANOVA followed by Tukey's test. *** indicates $P < 0.001$.

in response to 12 ppm vinegar (Fig. 17d), indicating that it is activity in the DM5 ORNs, rather than activation of a particular receptor, that biases the behavior toward aversion.

**4.5 Discussion**

It is a common feature of olfactory perception that most odors become less pleasant and eventually repellent as their intensity is increased [1], a phenomenon that has also been observed in *Drosophila* [5-7]. The recruitment of additional glomeruli has been proposed as a mechanism to mediate this change in behavioral output [2]. A recent paper has suggested that different levels of activation in the same ORNs could generate qualitatively different behavioral responses [3]. Here we found that a glomerulus recruited by a high concentration of vinegar, DM5, plays an important role in the behavioral switch. Silencing and selective activation experiments show that DM5 is necessary and sufficient for the behavioral switch.

The present results suggest that certain olfactory receptor neurons in *Drosophila* are genetically hardwired to generate robust innate olfactory attraction or avoidance behavior, an organizing principle that has been observed in several chemosensory systems [8-11]. In the fly, projection neurons receive input from ORNs and send axons to the mushroom body and lateral horn. Further studies should shed light on the mechanism by which these centers generate the behaviors we observe.

**4.6 Methods**.

**Odor stimuli.** The 12 ppm concentration of vinegar corresponds to 80 uL, 32 ppm is 1 mL vinegar, and 7 ppm ethyl butyrate came from 40 uL of a 1:1000 dilution of ethyl butyrate in mineral oil. The odor source was replenished for each experiment. Odor concentrations stayed constant over the time course of an experiment.

**Transgenic Flies.** The following fly stocks were used: *UAS-Or22a, UAS-Or83b*, *Or83b* targeted deletion ($Or83b^{2}$) [12]; *UAS-shibire*$^{ts}$ [13]; *UAS-GCaMP* [14]; *GH146-Gal4*[15].

This chapter, with chapter 3, was published in Nature in 2009 under the title "Select *Drosophila* glomeruli mediate innate olfactory attraction and aversion". The dissertation author was the primary author of this paper, with Jing Wang as co-author.

**References**


1. Laing, D.G., H. Panhuber, and R.I. Baxter, *Olfactory Properties of Amines and N-Butanol.* Chemical Senses & Flavour, 1978. **3**(2): p. 149-166.

2. Malnic, B., et al., *Combinatorial receptor codes for odors.* Cell, 1999. **96**(5): p. 713-23.

3. Kreher, S.A., et al., *Translation of sensory input into behavioral output via an olfactory system.* Neuron, 2008. **59**(1): p. 110-24.

4. Aurand, L.W., et al., *Volatile Components in Vapors of Natural and Distilled Vinegars.* Journal of Food Science, 1966. **31**(2): p. 172-&.

5. Stensmyr, M.C., et al., *Novel natural ligands for Drosophila olfactory receptor neurones.* J Exp Biol, 2003. **206**(Pt 4): p. 715-24.

6. Heimbeck, G., et al., *A central neural circuit for experience-independent olfactory and courtship behavior in Drosophila melanogaster.* Proceedings of the National Academy of Sciences of the United States of America, 2001. **98**(26): p. 15336-15341.

7. Wang, Y., et al., *Blockade of neurotransmission in Drosophila mushroom bodies impairs odor attraction, but not repulsion.* Curr Biol, 2003. **13**(21): p. 1900-4.

8. Troemel, E.R., B.E. Kimmel, and C.I. Bargmann, *Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in C. elegans.* Cell, 1997. **91**(2): p. 161-9.

9. Zhao, G.Q., et al., *The receptors for mammalian sweet and umami taste.* Cell, 2003. **115**(3): p. 255-66.

10. Suh, G.S., et al., *A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila.* Nature, 2004. **431**(7010): p. 854-9.

11. Kurtovic, A., A. Widmer, and B.J. Dickson, *A single class of olfactory neurons mediates behavioural responses to a Drosophila sex pheromone.* Nature, 2007. **446**(7135): p. 542-6.

12. Larsson, M.C., et al., *Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction.* Neuron, 2004. **43**(5): p. 703-14.

13. Kitamoto, T., *Conditional modification of behavior in Drosophila by targeted expression of a temperature-sensitive shibire allele in defined neurons.* J Neurobiol, 2001. **47**(2): p. 81-92.

14. Wang, J.W., et al., *Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain.* Cell, 2003. **112**(2): p. 271-82.

15. Stocker, R.F., et al., *Neuroblast ablation in Drosophila P[GAL4] lines reveals origins of olfactory interneurons.* J Neurobiol, 1997. **32**(5): p. 443-56.