Decoding olfaction in *Drosophila*

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Recent experiments in *Drosophila* demonstrate striking stereotypy in the neural architecture of the olfactory system. Functional imaging experiments in mammals and honeybees suggest a mechanism of odor coding that translates discrete patterns of activity in olfactory glomeruli into an odor image. Future experiments in *Drosophila* may permit a direct test of this odor-coding hypothesis.

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Abbreviations

AL	antennal lobe
DOR	*Drosophila* odorant receptor
OB	olfactory bulb
OR	odorant receptor
OSN	olfactory sensory neuron
PN	projection neuron

Introduction

Olfaction is a primitive sense that permits all animals to find food, identify conspecific mating partners, and avoid predators, and, allows insects to identify suitable substrates for egg-laying. The stimuli controlling these various olfactory-driven behaviors consist of blends of volatile organic chemicals that differ in size, shape, charge, and functional groups. For instance, the salient olfactory constituents of the rose consist of 275 distinct chemicals, whose ratio in the blend is carefully calibrated to produce its characteristic scent [1]. The enduring mystery of the olfactory system — unsolved to this day — is how the brain parses complex and often contradictory blends of odorous chemicals in the environment into meaningful odor images.

The anatomy of the olfactory system is well understood. In both vertebrates and insects, large numbers of functionally distinct primary olfactory sensory neurons (OSNs) extend dendrites that interact with odors from the external world. Odorants, the chemicals that compose odors, are recognized by distinct odorant receptor (OR) proteins that reside in the dendritic membrane. These receptor proteins, consisting of seven transmembrane domains, transduce odor recognition into neuronal activation through G-protein-coupled second messenger signaling pathways. Each OSN extends a single axon that synapses with second-order neurons in the olfactory bulb (OB) in vertebrates and the antennal lobe (AL) in insects. From this first olfactory synapse, information is relayed to higher brain centers, and ultimately to the descending motor pathways that drive appropriate behaviors.

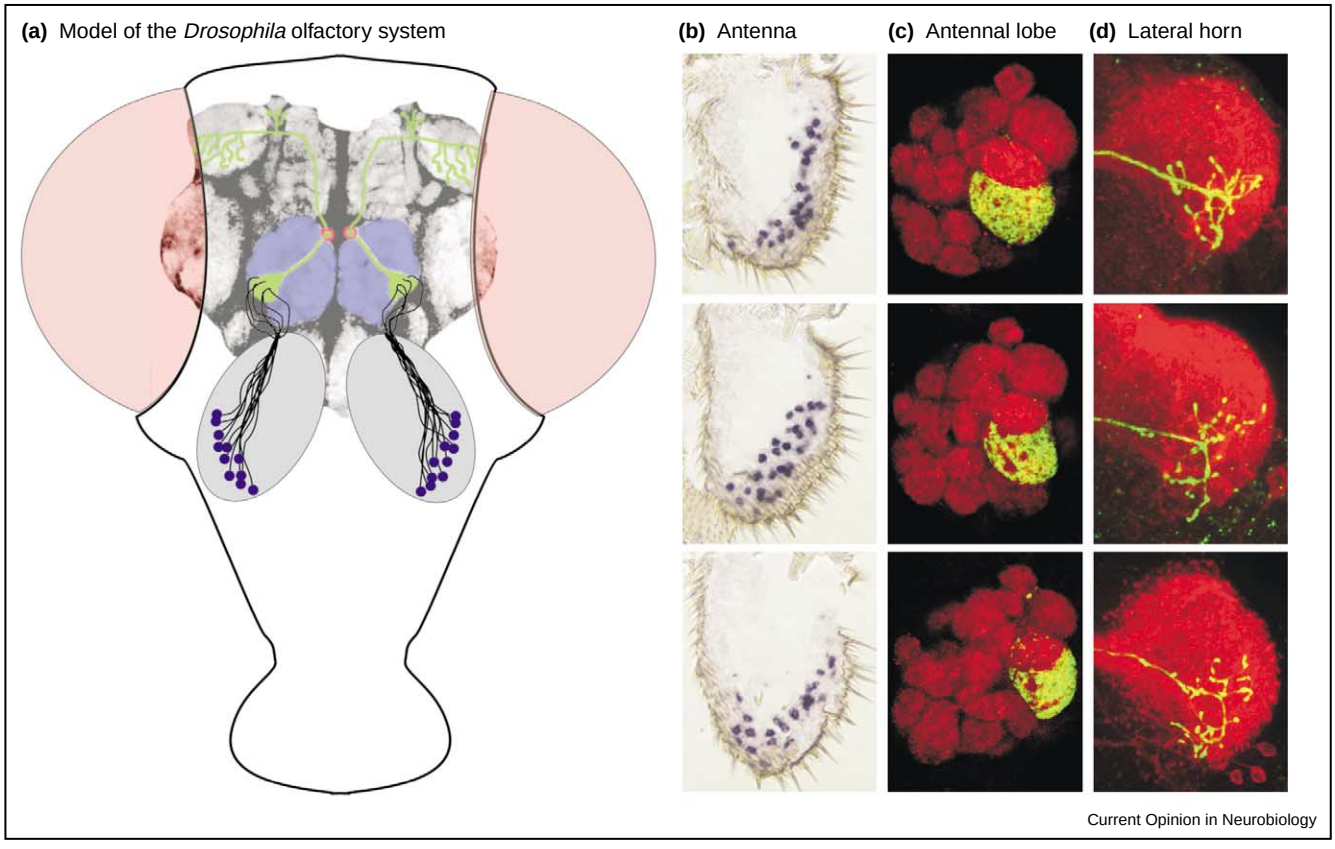
In this review, we discuss advances in understanding the link between the neural circuitry of the olfactory system and the mechanism that encodes odor images in the brain, with special emphasis on studies in the fruit fly *Drosophila melanogaster*. Recent functional expression experiments have proven conclusively that the candidate odorant receptors identified by molecular biology do indeed act as ligand binding proteins that transduce odorant-specific signals in the olfactory system. Odorant receptor genes are a rapidly evolving gene family, and this may account for variations in olfactory preferences among different species. In the fly, stereotypy has emerged as the organizational principle in wiring both first order and second order connections. Finally, functional imaging experiments, coupled with behavior paradigms suggest that the stereotypy in wiring may contribute to the process of encoding the odor image.

Evidence that *Drosophila* odorant receptors function in recognition

A large family of candidate odorant receptor (OR) genes in the rat was reported more than ten years ago [2]. Each OR gene encodes a different seven transmembrane domain G-protein-coupled receptor, which is expressed selectively in a subset of OSNs. Since this initial report, ORs have been identified in many vertebrate species (reviewed in [3]) and functional evidence that they bind odors and transduce olfactory-specific signals has been obtained in heterologous expression studies [4–6].

Odorant receptors in *Drosophila* are encoded by a distinct gene family, containing at least 61 genes, with no sequence similarity to vertebrate OR genes [7–9]. Each *Drosophila* odorant receptor (DOR) gene, with the exception of the broadly expressed receptor *Or83b*, is expressed in a small, positionally invariant subset of OSNs (Figure 1b). A second, distantly related gene family of gustatory receptors includes some members that are expressed in the olfactory system, although their role in olfaction has not yet been investigated [10,11].

Figure 1



Stereotypy in the labeled line of *Or47b* at all levels of the *Drosophila* olfactory system. **(a)** A frontal schematic view of a *Drosophila* head that depicts the olfactory circuitry displayed in Figure 1b–d. Dorsal is up; compound eyes are lateral and shaded red; antennae are medial and shaded grey; the central brain is visible through the head capsule; the AL is shaded blue. OSNs expressing *Or47b* are represented as blue dots on the antenna. These neurons extend axons (in black) that fasciculate and extend dorsally toward the brain and synapse in the VA1l/m glomerulus (shaded green) in the AL. The cell bodies of two dorsal PNs that synapse with *Or47b*-expressing OSNs in the AL are indicated in red. The dendrites of these cells innervate the VA1l/m glomerulus (large green structure in lateral AL) and the axons (in green) extend dorsally to make synapses both in the mushroom body calyx and the lateral horn. **(b)** Expression of the *Or47b* odorant receptor gene is restricted to a spatially conserved lateral-distal domain of the third antennal segment. **(c)** All axons from *Or47b*-expressing olfactory sensory neurons converge upon the VA1l/m glomerulus in the antennal lobe. **(d)** Axonal projections of a single dorsal group projection neuron, which sends dendrites to the *Or47b* (VA1l/m) glomerulus, are stereotyped in the ventral region of the lateral horn of the protocerebrum. Data in Figure 1(d) were kindly provided by Allan Wong, Jing Wang, and Richard Axel [30••].

Recent functional expression data confirm that DORs can recognize odorants. *Or43a* was found to encode a low-affinity receptor for cyclohexanol, cyclohexanone, benzyl alcohol, and benzaldehyde, both by heterologous expression in *Xenopus* oocytes and by overexpression in the *Drosophila* antenna [12••,13••]. Studies of the molecular receptive range of *Or43a* and previous analysis of rat OR I7 [14] suggest that a given OR can interact with several different odorants with varying affinity. A given odorant is also detected by more than one OR [15]. This receptor promiscuity would give rise to a combinatorial code of odors, such that an animal would be expected to detect many more odorants than the number of OR genes it possesses [15].

Rapid evolution of odorant receptor genes in insects

To what extent is odor recognition species-specific? Rapid divergence of OR genes is apparent in a comparison between two dipterans, *Drosophila* and the malaria mosquito *Anopheles gambiae*, and the moth *Heliothis virescens* [16••,17•]. *Anopheles* has 79 ORs and, with the exception of *Or83b*, no direct orthologue of any DOR gene is apparent in the *Anopheles* genome sequence. Phylogenetic analysis reveals large *Drosophila*-specific and *Anopheles*-specific OR subfamilies, which may subserve the recognition of the very different odors that are ecologically relevant to these dipterans. Similarly, the eight cloned *Heliothis* OR genes show a low degree of

sequence similarity to the OR gene families of the two dipterans [17•].

In marked contrast to this apparent species-specificity of all other insect ORs, *Or83b* is exceptionally well conserved from *Drosophila* to *Anopheles* to the moth *Heliothis virescens* [16••,17•]. This receptor is broadly expressed in most OSNs in *Drosophila*, such that each neuron expresses a conventional DOR along with *Or83b*. Whether *Or83b* functions as a heterodimer with conventional ORs to modulate ligand specificity, plays a role in receptor trafficking, or couples DOR activation to downstream signal transduction machinery is not known. However, the greater than 65% amino acid identity of *Drosophila*, mosquito, and moth *Or83b* suggests that this protein plays a central, well-conserved role in insect olfaction.

Stereotypy in wiring the olfactory system

Remarkably, the functional architecture of the olfactory system is similar in *Drosophila* and the mouse, despite the apparently independent evolution of the OR genes in these two species. In both mouse and *Drosophila*, neurons expressing a given OR gene extend axons that converge to form spatially discrete synapses with second-order projection neurons (PNs) (Figure 1). These synapses are organized into a spherical neuropil called a glomerulus, which consists of OSN axons, projection neuron dendrites, and input from a network of local inhibitory interneurons. Individual glomeruli therefore collect input of OSNs expressing a given OR gene (Figure 1c; [18–22]). Convergent wiring of neurons expressing the same OR, which therefore respond to the same odorants, may provide the basis for the brain to translate patterns of glomerular activity into perception of a stimulus.

By what mechanisms do these stereotyped and precise glomerular maps of OR axons form? Studies in the mouse have implicated the OR protein itself in the guidance process. Deleting an OR gene disrupts axon targeting, and replacement of a given OR gene with an alternate OR gene drives the axons to form synapses within an ectopic glomerulus [18,23]. Invoking the OR itself as a guidance molecule simplifies the problem of wiring millions of OSNs expressing one of several hundred different ORs. In fact, recent experiments that examined mice expressing ectopic OR genes suggest that these OSNs form ectopic glomeruli in the OB that are functionally innervated by mitral cells, the vertebrate PNs [24••,25•]. The formation of *de novo* functional glomeruli via OR-based axon guidance would permit the brain to cope with the rapid evolution of the OR gene family, enabling the generation of novel OSNs with new chemical specificities and novel synaptic connectivity in the OB.

Although direct evidence is lacking, it seems unlikely that a similar OR-dependent wiring mechanism operates to pattern glomerular connections in the *Drosophila* AL. *Drosophila* OSNs are born early in pupal life and extend axons and form glomeruli that are essentially complete by the mid-pupal stage [26]. However, the DOR genes are not expressed detectably until the end of pupal development [7,27]. The late-onset of OR gene expression in *Drosophila* suggests that OR-independent mechanisms establish the glomerular map.

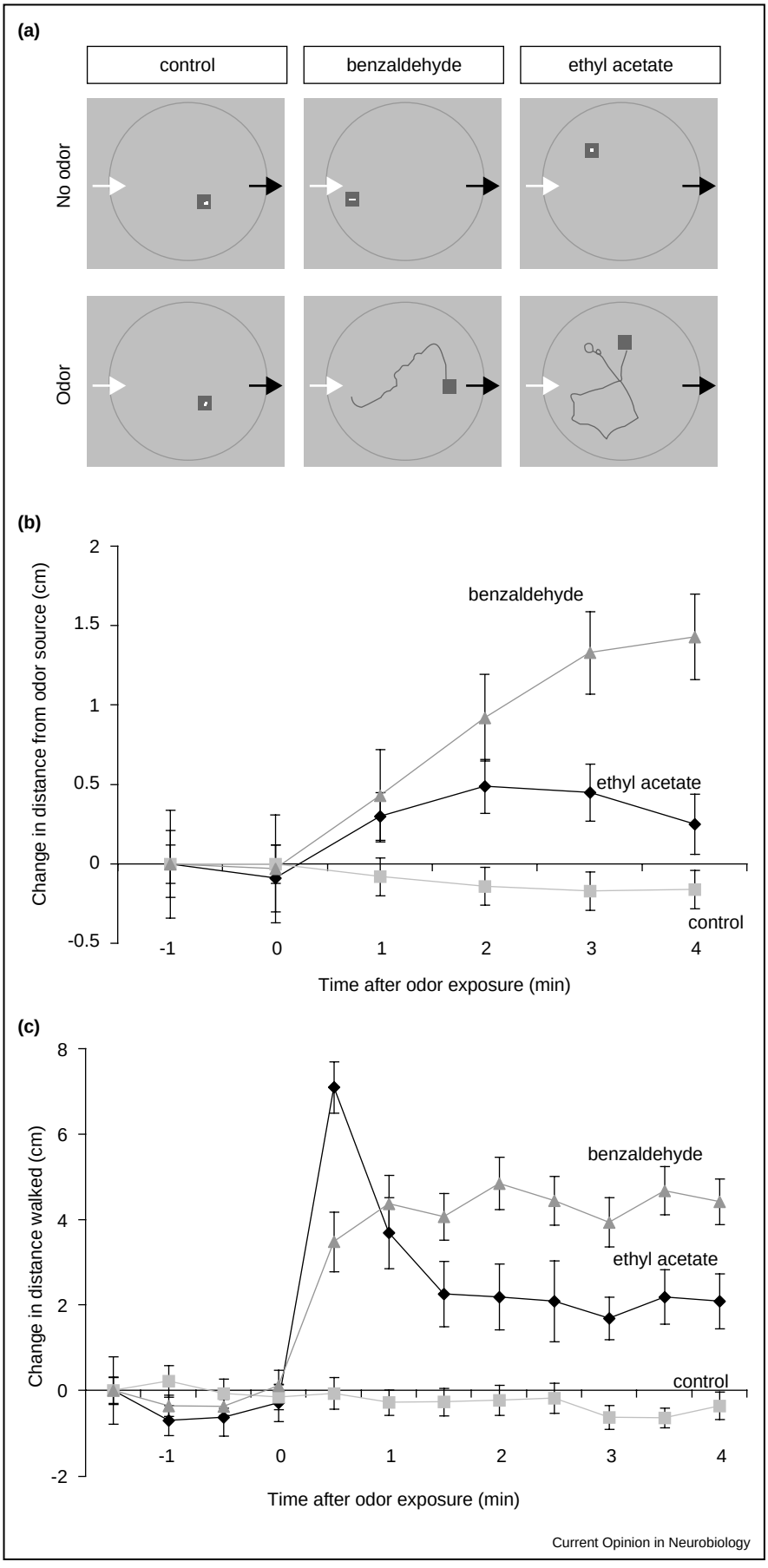
Olfactory information must be relayed from convergent synapses in the AL and OB to higher brain centers, where it is decoded to yield a coherent odor image. Recent work in *Drosophila* has produced the remarkable finding that the innervation of specific AL glomeruli by dendrites of a given second-order PN is invariant, and that these patterns are specified early in development before contact with OSNs and long before onset of DOR gene expression in these OSNs [28••]. Thus, it seems likely that independent pre-specification drives afferent OSN axons and postsynaptic PN dendrites to converge onto a given common glomerulus. This model appears to rule out any activity-dependent communication between OSNs and their targets in the AL that would serve to link up neurons by common functional properties, and instead requires a highly stereotyped genetic pre-programming of a neuronal circuit.

Similar stereotypy is seen in the axonal projections of PNs as they synapse with their targets in the mushroom body and the lateral horn of the protocerebrum (Figure 1d; [29••,30••]). PNs that innervate a given glomerulus have an invariant pattern of axonal branching in the lateral horn. They target diffuse and overlapping regions of the lateral horn. Thus, the convergent olfactory wiring seen in the AL is represented at higher levels of the brain as a widely distributed but spatially invariant map in the mushroom body and the lateral horn. Experiments in mice that traced the olfactory circuitry of OSNs expressing a given OR suggest a similarly distributed olfactory code in the vertebrate olfactory cortex [31•]. These tracing studies are consistent with functional studies that demonstrate a characteristic response property of individual mitral cells that reflects the molecular receptive range of the glomerulus from which it receives olfactory input [32,33].

Functional imaging techniques used to discern mechanisms of odor coding

The stereotypy apparent at all levels of the olfactory system (Figure 1) is highly suggestive of a mechanism of odor coding that employs spatial patterns of glomerular activation to represent olfactory stimuli, but direct proof of this model is lacking. Recent efforts to optically record olfactory glomeruli in diverse species confirm that the glomerulus is a functional unit in the olfactory system [34–38,39••,40•]. Using either intrinsic signal imaging or calcium-sensitive dyes, these investigators have shown,

Figure 2


(a)
control
benzaldehyde
ethyl acetate
No odor
Odor
(b)
Change in distance from odor source (cm)
benzaldehyde
ethyl acetate
control
Time after odor exposure (min)
(c)
Change in distance walked (cm)
benzaldehyde
ethyl acetate
control
Time after odor exposure (min)
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first, that a given odorant activates a reproducible subset of glomeruli that is invariant between different individuals of a species; second, that with increasing concentration additional glomeruli are recruited into the activity pattern, and third that glomeruli responsive to chemically related odorants are clustered on the surface of the vertebrate OB. The most recent direct proof that signals obtained in functional imaging represent OR-specific glomerular input was provided by experiments in genetically manipulated mice that express ectopic OR genes in fluorescently tagged OSNs [24••,25•]. Experiments in the honeybee and the moth [41•,42•] that examined information flow in the AL suggested that inhibitory interneurons play a major role in modulating the output of glomerular activity. The inhibitory network filters and processes the olfactory information that arrives at the glomeruli from the OSNs and produces a coherent stimulus-specific output.

How is olfactory information from the AL or OB represented at higher levels in the brain? The PN tracing studies in *Drosophila* suggest that although PNs form highly distributed synapses in the mushroom body and lateral horn, the patterns of synapse distribution are strongly conserved between different individuals. Electrophysiological experiments in the locust and the zebrafish carried out by Laurent and co-workers [43••,44] have led to an alternate hypothesis of odor coding that does not rely solely on the hypothesis of glomerular encoding and instead favors a temporal model. In this model, odor stimulation induces stimulus-specific alterations in the synchrony of local field potentials. It is proposed that these temporal parameters are central to the process of representing odorants, especially those that are structurally similar and likely to produce overlapping patterns of activation in the OB or AL.

Behavioral discrimination and the glomerular code

Ultimately, determining how odors are encoded in the brain will require linking a specific behavioral output with an olfactory input, and correlating this link with a measure of synaptic activity in the brain. In practice, this can be done by testing the ability of animals to discriminate between odors. Behavioral experiments in the moth *Manduca sexta* showed that this species can discriminate between similar odors, although there is overlap in the representation of these odors [45•]. Furthermore, both honeybees and rats can efficiently discriminate enantiomeric pairs of odorants, that is, chemicals that differ only in their left/right handedness [46,47]. Functional imaging of the responses of the rat OB in response to these stimuli revealed activation of largely overlapping, yet different, patterns. This is consistent with the rat's ability to discriminate these odors behaviorally. It seems that enantiomeric pairs with very similar activation patterns are only discriminated after reinforcement, whereas the pairs evoking less similar activation patterns are distinguished spontaneously [48••,49••]. A learning-dependent effect on glomerular activity in the honeybee AL was described several years ago [50], and illustrates the reciprocal dependence of behavioral output and synaptic activity in the brain. Pharmacological perturbations that affected both the local field potential oscillation and the modulation of PN output by local inhibitory interneurons caused honeybees to lose the ability to discriminate between closely related odorants, although they retained their ability to discriminate distinct stimuli [51]. Many of these experiments provide a good correlation between patterns of glomerular activity and the animal's behavioral performance. However, they stop short of demonstrating that these activity patterns are the salient information that the animal uses to encode the odor. *Drosophila* provides a unique system that may permit the linking of mechanisms that control the development of olfactory circuitry to an understanding of how this circuitry serves to generate and organize complex behaviors.

Conclusions

Despite the diversity of olfactory responses among different species, glomerular coding of olfactory stimuli appears to be a central and conserved mechanism. In *Drosophila* and mice, the wiring of OSNs to the first olfactory relay is highly stereotyped and leads to a convergence of all of the neurons expressing a given OR gene to one or two glomeruli. The majority of the PNs extend dendrites into a single glomerulus and elaborate axonal processes that terminate in stereotyped patterns in the mushroom body and lateral horn of the fly. Finally, flies exhibit robust olfactory-driven behaviors that reveal striking differences in the responses of a single fly to distinct odorants (see Figure 2). By utilizing the genetic tools available in *Drosophila*, it should be possible to silence or activate distinct parts of this simple olfactory circuit and to monitor the resulting behavioral output. This approach may reveal new insights into the mechanisms by which

Figure 2 Legend Behavioral responses of *Drosophila* to odors. **(a)** Tracks of individual flies in a circular arena measured by videography coupled to tracking software. Left arrows depict the site of odor input and right arrows odor output. 30 s tracks of three flies before (above) and after (below) onset of odor delivery are shown. Both benzaldehyde and ethyl acetate but not pure air induce activity in resting flies. The squares represent the position at the end of the 30 s assay period. **(b)** The change in distance from the source of ethyl acetate and benzaldehyde. The avoidance of benzaldehyde is more pronounced than the avoidance of ethyl acetate. **(c)** The increase in activity after onset of odor exposure. Both benzaldehyde and ethyl acetate induce activity. However, the temporal dynamics of these activities differ. Ethyl acetate induces a high initial activity that decreases after 30 s to reach a plateau, whereas benzaldehyde induces a slow increase in activity that reaches a plateau higher than the one seen for ethyl acetate activity. Means of 50 to 60 flies are given. Error bars indicate SEMs.

organisms respond to the odorous environment in which they live.

Update

Three recent papers report the shattering of a technical barrier that has prevented the imaging of synaptic activity in the AL of living *Drosophila* while they are exposed to odors [52••–54••]. The small size of *Drosophila* and the relatively low number of OSNs converging upon a given glomerulus in the AL have precluded the use of conventional optical imaging techniques that employ Ca^{+2}-sensitive dyes or intrinsic signal imaging. Both groups overcome the problem of size and signal strength in *Drosophila* by the use of genetically encoded sensors of neuronal activity, which permit the selective expression under genetic control of high levels of fluorescent sensor proteins in the neurons of choice in the olfactory network.

Ng and co-workers [52••] used synapto-pHluorin, which is a pH-sensitive variant of GFP that is tethered to the synaptic vesicle and produces pH-dependent fluorescence changes upon evoked synaptic release. They examine odor-evoked synaptic activity simultaneously in OSNs, PNs, and inhibitory interneurons in the AL, by selective expression of synapto-pHluorin in these different cell populations. Similar to previous studies in the honeybee, the *Drosophila* responses showed a combinatorial logic, with odors activating distinct but overlapping glomeruli in the AL. These responses were highly reproducible between different individuals and the number of glomeruli activated increased with increasing concentrations of odorant. Unlike the honeybee, in which activity patterns elicited by a given odorant were broader when measured in OSNs than postsynaptic PNs [41•], *Drosophila* responses appeared to be highly similar whether measured in OSNs or PNs. In contrast, local interneurons demonstrated much broader and more complex patterns of activation.

Fiala *et al.* [53••] used cameleon as a genetically encoded Ca^{2+} sensor to monitor odor-evoked changes in the activity of *Drosophila* PNs in the AL and in the calyx of the mushroom body. They find a similar degree of stereotypy in activation of discrete foci in the AL — which are likely to be glomeruli — and show for the first time that spatially conserved regions of the mushroom body calyx are activated in response to a given odorant. These results are significant because they are a functional correlation of the stereotypy in PN axonal connectivity demonstrated by the Axel and Luo groups (Figure 1d; [29••, 30••]).

Finally, Wong *et al.* [54••] use a third genetically encoded calcium sensor protein, G-CaMP, which produces fluorescent intensity changes of up to 120%, to characterize the response properties of 23 glomeruli to 16 different odorants. They find that the glomerular code is sparse at low stimulus concentrations and that at higher concentrations, a large number of glomeruli are recruited. They argue that the sparse odor code is more likely to represent the physiological state of the animal, than the highly overlapping promiscuous code obtained at high stimulus concentrations. Comparison of activity in OSNs and PNs suggests that information is relayed faithfully, with minimal processing or filtering, to higher brain centers. In a genetically reprogrammed fly, the activity of a glomerulus is shown to be a property of the OR gene expressed in OSNs that synapse in that glomerulus.

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