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Internal representations of smell in the *Drosophila* brain

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Abstract

Recent advances in sensory neuroscience using *Drosophila* olfaction as a model system have revealed brain maps representing the external world. Once we understand how the brain's built-in capability generates the internal olfactory maps, we can then elaborate how the brain computes and makes decision to elicit complex behaviors. Here, we review current progress in mapping *Drosophila* olfactory circuits and discuss their relationships with innate olfactory behaviors.

Introduction

All animals are born with *a priori* capabilities that are hardwired into the nervous system for instinctive behaviors independent of experience. The internal representation of the external world is then evolved as a result of the brain's built-in capability to derive meaning from information in the surrounding environment. A cognitive map is therefore established through the experience-dependent modifications in the prewired brain circuits. To elicit adaptive behavioral responses, the spatiotemporal pattern of sensory stimuli perceived by an animal must be ultimately translated into neural codes in the brain. How information of the outside world is transmitted to the inside of the brain? This big question has been extensively addressed in a small but functional neural circuit: the olfactory system of the fruit fly, *Drosophila melanogaster*. *Drosophila* exhibits many complex behaviors controlled by an olfactory system that has been proven to be genetically and anatomically similar to those in the vertebrates [1–5]. Amenability of the genetic toolbox in the *Drosophila* allows manipulation of identified brain neurons with such a spatial and temporal precision that results in unprecedented insight into the design and function of the brain. Advances in *Drosophila* sensory neuroscience are clarifying information processing mechanisms that underlie a response in the brain to a stimulus [6]. Here, we limit our discussion on the current progress in mapping brain circuits involved in the *Drosophila* olfactory behaviors. Understanding the neural maps representing olfaction will have a great impact on the insight of molecular and cellular mechanisms involving in the olfactory memory formation [7, 8].

Organization of the *Drosophila* olfactory circuitry

Figure 1 shows the organization of the *Drosophila* olfactory circuitry. Odors are detected by a large family of odorant receptors (ORs) expressed in

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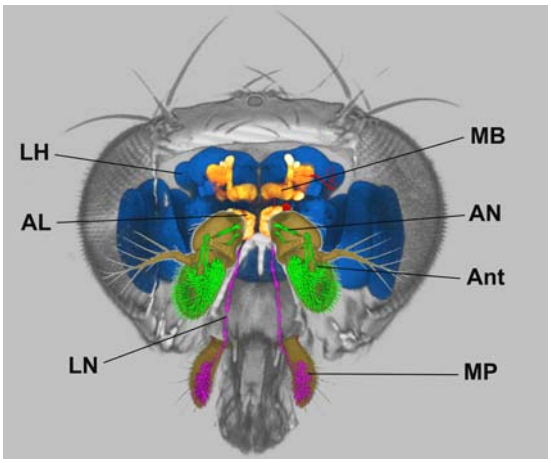

Figure 1. Organization of the *Drosophila* olfactory system. Odorants are detected by the olfactory sensory neurons (OSNs) located on the antenna (Ant, green) and maxillary palp (MP, magenta) sending their axons via the antennal nerve (AN) and the labial nerve (LN), respectively, to the antennal lobe (AL, orange); where the projection neurons (PNs, red) receive the information and relay to the mushroom body (MB) and the lateral horn (LH). This picture is a montage of four sets of confocal images taking from the whole head (grey), brain (blue), antenna (Ant), and maxillary palp (MP) separately from different flies. The head image is derived from auto-fluorescence excited with 633 nm laser. MB and AL (orange) are segmented from the whole brain stained by nc82 antibody. OSNs in the antenna and MPs are labeled by OR83b-GFP. The single PN image is derived from MARCM visualization of GH146-GAL4.

olfactory sensory neurons (OSNs) on the maxillary palp and the antenna [9]. Individual OSNs express only one, or a few, but rarely, OR on the ciliated endings of OSN dendrites [10–12]. The axons of OSNs expressing the same OR converge to a single glomerulus in the ipsilateral antennal lobe (AL) [13–15], where they synapse with projection neurons (PNs) that project to the mushroom body (MB), the lateral horn (LH) [9] and inhibitory and excitatory local neurons (LNs), that might modulate the information among glomeruli [9, 10, 16].

The OR-to-OSN map

Drosophila has a family of 60 OR genes that encode 62 odorant receptors determining the odor-response profile of the OSNs [14, 17–20]. Each OR gene expresses in a subset of OSNs except Or83b that acts as a co-receptor in ca. 70% OSNs [20–23]. Flies lacking OR83b display severe defects in many different olfactory behaviors but with their lifespan extended [22, 24]. In *Drosophila* larva, each of the paired olfactory dorsal organs houses 21 OSNs. Each OSN expresses a single OR in addition to OR83b, including 11 ORs that are not expressed in the adult [25–27]. In *Drosophila* adult, the recognition of odors is accomplished by sensory hairs distributed over the surface of the third antennal segment and the maxillary palp. There are about 2600 OSNs housed inside three different types of hair-like sensilla: the club-shaped basiconic, the long-pointed trichoid and the short coeloconic sensilla [28–31]. The OSNs housed inside the distal-lateral trichoid and the proximo-medial basiconic sensilla project their axons to lateral-anterior and proximo-medial AL glomeruli, respectively [32–34]. In the maxillary palp, OSNs projecting via the labial nerve to the AL are all contained in the basiconic sensilla. Every individual flies has about 1000 sensilla, each contains 1–4 OSNs [35]. The bipolar OSNs project dendrites in sensillar lymph interacting with odorants and project axons to the AL, where olfactory information is processed. In the sensillar lymph, olfactory binding proteins secreted by the support cells may act as transport vehicles for the odorants to bind with the OR. Odorant-OR binding then activates a heterotrimeric G-protein that influence the action potentials transmitted via axons to the AL [36–38]. Intriguingly, a single ORN can exhibit both stimulatory and inhibitory responses to different odorants [39]. A chemotopic map of OR responses to 110 odorants indicates that OSN responses are chemical class-dependent [39, 40].

The OSN-to-PN map

In the ALs, the axons of OSNs project to about 50 glomeruli, where they synapse with dendrites of intrinsic LNs and output PNs. Axons of OSNs with the same OR expression converge to the same AL glomerulus suggesting stereotypic connectivity with PNs [15, 41–43]. Co-convergence to VA6 glomerulus from two different classes of OSNs labeled by different OR Gal4 lines is an exception [32, 33]. Therefore, a topographic map of OR responses in the peripheral sensory organs is further represented in the ALs [44]. Stereotypic connectivity between the OSNs and PNs has been confirmed by functional calcium imaging [20] and 2-deoxy glucose mapping [45, 46] showing that

different odorants elicit defined glomerular activities. In the ALs, odor responses of the PNs are modulated by local neurons that form widespread intra- as well as inter-glomerular connections among many glomeruli [16, 47–50]. It has been proposed that odor coding is sharpened at ALs by GABAergic LNs that inhibit background signals from adjacent glomeruli [49]. However, this can not explain why PNs are more broadly tuned to different odors than OSNs [20]. A newly discovered population of cholinergic LNs with multiglomerular processes responds broadly to odors [16] suggesting that PNs receive information from a combination of glomerulus-specific OSN afferents and "diffused" LN excitation [16].

The PN-to-LH map

PNs convey olfactory information from paired ALs to high brain centers, MB and LH, via three different tracks: the inner antenna-cerebrum track (iACT), the medial ACT (mACT), the outer ACT (oACT). One AL has about 50 glomeruli, each innervated by dendrites of 3–7 PNs [44, 51, 52]. As a general principle, one PN sends dendrites into only a single glomerulus. PN axons may directly project to LH via mACT and oACT. Most PNs, however, project in parallel via iACT to the LH en route MB calyx. A typical PN usually gives 1–4 branches in the calyx and has many termini in the LH. How do the PNs carry olfactory information to the high brain centers? Using genetic mosaic FLP-out technique, visualization of individual PNs has revealed that the axonal branch patterns of PNs coming from the same AL glomerulus display stereotypy in the LH among different flies [51, 52]. These axonal termini of PNs in the LH appear to segregate into three distinct spatial domains: anterior ventral, posterior dorsal and lateral ventral regions [53]. Visualization of gene expression patterns from more than 4000 GAL4 lines have revealed three different classes of LH local neurons that have projection patterns corresponding to the distribution of PN axonal termini [53]. These results suggest a stereotypic connectivity between specific PNs and specific LH neurons via iACT and mACT. If so, a topographic map of olfactory responses in the ALs is further represented in the LH [53]. It has not been addressed if PNs via oACT also give stereotyped termini in the LH and if their types are different from those via iACT and mACT.

The PN-to-KC map

The spatial distribution of PN axon branching patterns is highly stereotyped in the LH but less clear in the MB [51, 52]. The paired MBs play an essential role in insect olfactory behaviors [54]. One MB in adult *Drosophila* consists of ~2500 intrinsic Kenyon cells (KCs) derived from four neuroblasts [55, 56], each of which sequentially generates five distinct classes of KCs: γ, $\alpha'\beta'$, pioneer $\alpha\beta$, early $\alpha\beta$ and late $\alpha\beta$ respectively [57, 58]. KCs give dendrites exclusively in the calyx, where they synapse with PN axonal termini. KC axons constitute a massive parallel tract called peduncle that extends ventral-frontally and splits at its distal end: one projecting dorsally and the other medially. The dorsal projection is composed of α and α' lobes, and the medial projection is composed of β, β' and γ lobes [59]. Stereotyped odor-evoked activity occurs both in the AL glomeruli [20, 60] and the MB calyx [61]. As odor concentration increase, more glomeruli are activated in the AL and more KCs are activated in the calyx [20, 61]. PN axonal termini appear to form three concentric zones in the MB calyx [53]. Analysis of topographic connectivity between PNs and KCs in the MB calyx has been hampered due to insufficient optical resolution and lack of anatomical landmarks. The dual MARCM technique allowing visualization of individual PNs and KCs in two different colors may help to answer this question [62].

Olfactory information processing for innate behaviors

Innate behaviors, such as courtship ritual, are instinctive actions of an animal without prior experience. These inherited programs offer a unique opportunity to use genetic and anatomic analysis to dissect and characterize the neural substrates of complex behaviors. In insects, avoidance or attraction to different odors is essential for the exploitation of food sources, selection of mates and escaping from harmful situations. A central question in olfaction is how the brain discriminates

different odors to elicit an appropriate behavioral response. In *Drosophila*, stereotypic connectivity maps of OR-to-OSN [39], OSN-to-PN [32, 33], PN-to-LH [51, 52] and PN-to-KC [61] begin to reveal a hierarchy of information processing with increasing convergence, which enable higher-order centers to integrate information relayed separately from lower-order neurons. Although stereotypic connectivity maps from OSNs to PNs to KCs give the impression of a straight and simple path, neural computation for odor discrimination clearly requires multiple steps of information integration as it makes its way through the brain. A single OSN can exhibit both excitatory and inhibitory responses to different odorants [40]. In the ALs, odor responses of the PNs are modulated by inhibitory as well as stimulatory local neurons [16, 50]. In the MBs, KCs may receive both stimulatory and inhibitory stimuli from PNs since some of them are cholinergic but others are GABAergic [49]. Immunohistochemical labeling and GFP expression patterns in Cha-GAL4 and GAD-GAL4 lines indicate that KCs are also composed of both cholinergic and GABAergic neurons (Lin and Chiang, unpublished observation). Such complexity of odor representations and information integration hierarchies greatly reduces the possibility of overlap between spatiotemporal patterns elicited by two different odorants, making them easier to be discriminated or memorized and recalled [63].

A *Drosophila* uses CO_2 released by other stressed flies as an alarm signal [64]. CO_2 is sensed by antennal OSNs via a pair of chemosensory receptors, Gr21a and Gr63a [65]. CO_2-responsive neurons relay information to only a single glomerulus in the AL, the V glomerulus [64]. Inhibition of synaptic transmission in the CO_2–responsive neurons, using a temperature-sensitive *shibire* gene, blocks the avoidance response to CO_2. It remains unclear how the brain sense and apply the CO_2–induced signals in computation. Chemical ablation of the MBs abolishes olfactory attraction but not repulsion behaviors suggesting that the computation center for the attractive odors is likely at the MB and for the repulsive odors is likely at the LH [66]. Consistently, blocking neurotransmitter release from MBs using the temperature sensitive *shibire* has no effect on CO_2 avoidance behavior [64], implying that LH may involve in computing the CO_2–induced signals. However, this hypothesis remains to be verified since PNs connecting V glomerulus to higher brain centers have not been mapped and LH specific drivers are still unavailable for the manipulation of its biological functions.

Drosophila courtship represents the most complex innate behavior. A courting male performs a stereotypical sequence of ritual activities beginning with attracting to a receptive female and then he orients, taps, sings, licks and finally bends his abdomen to copulation [67]. Males exhibit homosexual courtship if a transcription factor gene called *fruitless* (*fru*) has defect. Although the roles of *fru* remain unclear, neurotransmission from brain neurons expressing *fru* is essential for normal courtship behavior [68]. Intriguingly, *fru*-expressing neurons appear to interconnect with each other, as indicated by that *fru*-expressing OSNs and PNs innervate the same set of AL glomeruli (VL2a, DA1, VA1 and VA6). *fru* expresses also in the MBs of the γ and α/β lobes, and courtship conditioning is impaired when the expression of male-specific *fru* transcript is disrupted in MB γ neurons [69]. A recent study indicates that *fru* is also associated with patterns of aggressive behavior that is sex-specific and with the formation of dominance rank in a group of flies [70]. These finding suggest that, aggression and mating are closely intertwined in *Drosophila*, as also in mammals [71, 72].

Concluding remarks

How the olfactory circuitry executes various different programs of innate behaviors? Olfactory information converges from OSNs to PNs in the AL and then diverges from PNs to KCs of the MB and principle neurons of the LH suggesting that information processing must perform in the AL to translate peripheral odor stimuli into a neural coding intelligible to neurons of high brain centers. Where the MB and LH send the processed information to execute specific behavior outputs? Are there other higher brain centers involving olfactory information processing? Screening mutants with defects in olfactory behaviors have identified a set of genes involving in particular olfactory innate behaviors [65, 68]. Mapping the expression of these olfactory behavior essential genes should reveal additional, if any, brain

circuits involving in the computation of olfactory information. Each step of information processing in the hierarchy can then be characterized by the manipulation of neuronal activities and gene expressions with various genetic tools available. Identification of genes (intracellular hierarchy) and circuits (intercellular hierarchy) involving in particular olfactory behaviors promises to reveal the secret of the brain's operations in the information acquisition, processing, transmission, storage, and retrieval.

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