Topographic Mapping—The Olfactory System

Takeshi Imai^{1,2}, Hitoshi Sakano¹, and Leslie B. Vosshall³

¹The University of Tokyo, Graduate School of Science, Department of Biophysics and Biochemistry, Yayoi 2-11-16, Bunkyo-ku, Tokyo 113-0032, Japan

²PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho Kawaguchi, Saitama 332-0012, Japan

³Laboratory of Neurogenetics and Behavior, Howard Hughes Medical Institute, The Rockefeller University, New York, New York 10065

Correspondence: leslie@mail.rockefeller.edu

Sensory systems must map accurate representations of the external world in the brain. Although the physical senses of touch and vision build topographic representations of the spatial coordinates of the body and the field of view, the chemical sense of olfaction maps discontinuous features of chemical space, comprising an extremely large number of possible odor stimuli. In both mammals and insects, olfactory circuits are wired according to the convergence of axons from sensory neurons expressing the same odorant receptor. Synapses are organized into distinctive spherical neuropils—the olfactory glomeruli—that connect sensory input with output neurons and local modulatory interneurons. Although there is a strong conservation of form in the olfactory maps of mammals and insects, they arise using divergent mechanisms. Olfactory glomeruli provide a unique solution to the problem of mapping discontinuous chemical space onto the brain.

The olfactory system detects airborne organic and inorganic chemicals that originate from plant and animal metabolites and environmental and industrial sources. Odors mediate both innate and learned behaviors such as attraction and aversion, governing decisions to eat, mate, attack or flee from aggressors and predators. A remarkable feature of the olfactory system is the extraordinary diversity of possible odor molecules that exist. Although accurate measurements can be made of the detection range of visual wavelengths or auditory frequencies in humans, there are no reliable estimates of the number of odorous molecules that exist on earth or those that can be detected by a given

animal species (Gilbert 2008). Nevertheless, the number of possible odorants is thought to be high, in the range of many thousands. Beyond odor detection lies the problem of odor discrimination: how can chemicals with slightly different physical properties be discriminated and associated with distinct odor percepts?

Experiments investigating the molecular logic of olfaction over the last 20 years have led to major insights into how animals solve the problem of detecting and discriminating a vast number of different odor stimuli (Axel 1995). Four organizational principles have emerged from this work. First, large numbers of distinct odorant receptor (OR) genes are dedicated

T. Imai et al.

to olfaction: ~ 1000 in mammals and ~ 100 in insects. Second, each olfactory sensory neuron (OSN) typically expresses only a single receptor out of this large repertoire. Each OR has a distinct odor ligand profile, such that there are ~ 1000 types of OSNs in the mouse and ~ 100 types in the typical insect. Third, OSNs expressing the same receptor extend axons that converge on the same glomerulus in the brain, forming a map of ORs in the brain. Fourth, each odor is encoded in a combinatorial manner: One odor can activate multiple ORs, and each OR can respond to multiple odors (Malnic et al. 1999; Hallem and Carlson 2006). As a result, odor information is spatially encoded in the first relay station of the brain (reviewed in Mori et al. 2006). Strikingly, these four principles along with the anatomical organization of the olfactory system are quite similar in mammals and insects, suggesting a mechanism of convergent evolution (Hildebrand and Shepherd 1997).

Recent studies in two genetic model organisms, the mouse and the fly, *Drosophila melanogaster*, have elucidated mechanisms governing how the topographic yet discrete olfactory map is established in the brain (see Luo and Flanagan 2007 for an excellent recent review). Although mice and flies have multiple olfactory subsystems and additional classes of chemosensory receptors (reviewed in Brennan and Zufall 2006; Touhara and Vosshall 2009), this article will focus on comparative mechanisms used to map ORs and OSNs from the main olfactory epithelium in the mouse and adult chemosensory structures in the fly to glomerular targets in the brain.

dendrite gives rise to 20–30 ciliary processes whose membranes contain OR proteins. Mammalian ORs are seven transmembrane G protein-coupled receptors (GPCRs) and were first discovered in rodents in 1991 (Buck and Axel 1991). The number of functional ORs varies widely across mammalian species from ~ 1000 in mice (Zhang and Firestein 2007) to ~ 400 in humans (Glusman et al. 2001).

Insect OSNs are anatomically similar to mammalian OSNs but are housed in specialized sensory hairs called sensilla that decorate the surface of the major olfactory organ, the antenna (Stocker 1994) (Fig. 2A,B). Insect ORs are seven transmembrane proteins that are evolutionarily distinct from mammalian ORs (Benton et al. 2006; Wistrand et al. 2006). The total number of such ORs in recently sequenced insect genomes varies from ~ 60 in *Drosophila* (Robertson et al. 2003) to ~ 260 in the red flour beetle *Tribolium* (Engsontia et al. 2008). Insect ORs are rapidly evolving and show little sequence similarity even within a given insect species. A single member of the insect OR super family, in contrast, is strongly conserved in all sequenced insect genomes (Krieger et al. 2003; Pitts et al. 2004; Jones et al. 2005). This gene, called Or83b in *Drosophila*, does not interact with odors but functions as a chaperoning coreceptor that assembles into a heteromeric complex with the ligand-binding ORs (Larsson et al. 2004; Nakagawa et al. 2005; Neuhaus et al. 2005; Benton et al. 2006). Thus although mammalian and insect OSNs are anatomically similar, the OR repertoire is smaller in insects than mammals and insect ORs are evolutionarily unrelated to vertebrate ORs.

PERIPHERAL OLFACTORY ORGANIZATION AND ODORANT RECEPTOR GENE CHOICE

Sensory Neurons and Odorant Receptor Proteins in Mammals and Insects

In the main olfactory system of mammals, inhaled odorants are dissolved in the olfactory mucosa in the main olfactory epithelium (OE) (Fig. 1A). OSNs in the OE extend a single dendrite into the lumen of the nasal cavity. The

Divergent Olfactory Signaling Mechanisms in Mammals and Insects

The binding of odor ligands to ORs in both mammals and insects activates a signaling cascade that results in depolarization of the OSN, but very different mechanisms activate mammalian and insect ORs. Mammalian ORs use canonical GPCR signaling to convert odor binding to neuronal depolarization. (reviewed in Ma 2007). OR signal transduction begins

Comparative Views on Olfactory Axon Guidance

Figure 1. Olfactory sensory neuron projections along the dorsal–ventral axis in the mouse olfactory system. (A) Lateral view of a *MOR28-IRES-tau-lacZ* transgenic mouse (Serizawa et al. 2000) stained with X-gal to reveal OSNs in the OE and *tau-lacZ*-tagged *MOR28* axons projecting to a specific site forming a glomerulus in the OB. (B) OSNs in the dorsomedial zone (D zone) in the OE project their axons to the dorsal domain (D domain) of the OB. Class I ORs are mostly expressed by OSNs in the D zone in the OE, which target the anterodorsal cluster of the D domain in the OB (red). In the ventrolateral zone (V zone), each class II OR possesses its own unique expression area, which is distributed in a continuous and overlapping manner along the dorsomedial–ventrolateral axis in the OE. The dorsomedial–ventrolateral expression area in the OE corresponds to the glomerular positioning along the dorsal–ventral axis in the OB. Thus, the dorsal–ventral arrangement of glomeruli is roughly determined by the locations of OSNs in the OE. Axonal projection along the dorsal–ventral axis is determined by axon guidance molecules such as *Nrp2*, *Sema3F*, and *Robo2* expressed in a graded manner in the OE. OSN, Olfactory sensory neuron; OE, olfactory epithelium; OB, olfactory bulb; OR, odorant receptor; D zone, dorsal zone; V zone, ventral zone.

T. Imai et al.

Figure 2. Functional connectivity in the *Drosophila* antennal lobe. (A) False-colored scanning electron micrograph image of a *Drosophila* head illustrating the major olfactory (antenna) and gustatory (proboscis) organs (photo: Jürgen Berger, Max Planck Institute for Developmental Biology, Tübingen, Germany). (B) Cross section of adult antenna hybridized with an in situ probe to reveal expression of *Or22a* mRNA. (C) Projection of *Or22a*-expressing axons to the DM2 glomerulus in the antennal lobe, as traced with n-synaptobrevin:GFP (green). Neuropil is counterstained with nc82 (red). (D) Connectivity map of the adult fly antennal lobe, with glomeruli color-coded according to OSN type at the periphery. The position of the *Or22a* glomerulus is marked by the arrow in panels (C,D). The antennal lobe is represented as four slices arrayed from anterior (A) to posterior (P), with glomerular position depth-coded such that white glomeruli are superficial, gray glomeruli intermediate, and black glomeruli deep.

with the heterotrimeric guanine nucleotide binding proteins $G_{\alpha s}$ and $G_{\alpha olf}$, which stimulate adenylyl cyclase type III (ACIII), leading to cAMP production and the gating of a calcium permeable, tetrameric cyclic nucleotide gated (CNG) channel, which contains CNGA2 as an essential subunit. $G_{\alpha olf}$, ACIII, and CNGA2 knockout mice are all anosmic (Brunet et al.

1996; Belluscio et al. 1998; Wong et al. 2000). Calcium influx through the CNG channel results in the opening of the chloride channel, ANO2 (Stephan et al. 2009), and the efflux of chloride, which depolarizes the OSN.

Insect ORs have seven transmembrane domains and although they lack sequence homology to mammalian ORs, they were generally

assumed to be GPCRs like mammalian ORs. Recent studies have questioned this assumption. Touhara and coworkers showed that expression of heteromeric insect olfactory receptors in heterologous cells was associated with an odor-gated cation current (Nakagawa et al. 2005). Further experiments that investigated the source of these currents led two groups to propose that insect ORs are odor-gated cation channels (Sato et al. 2008; Wicher et al. 2008), although the extent to which these channels are modulated by G-protein signaling remains controversial (Nakagawa and Vosshall 2009).

Odorant Receptor Gene Choice: Stochastic in Mice, Deterministic in Flies

In both mammals and flies, OR gene expression is tightly regulated to ensure that one (or in insects rarely more than one) ligand-binding OR is expressed per OSN. Such exclusion ensures that a given OSN has a limited range of chemical specificities. Interestingly, strict regulation to limit gene expression to one OR per OSN is achieved by entirely different mechanisms in mice and flies.

In the mammalian olfactory system, each OSN expresses only one OR from a large repertoire (Malnic et al. 1999) and expression is limited to either the maternal or paternal allele (Chess et al. 1994). This monogenic and mono-allelic mode of receptor gene choice is known as the “one neuron—one receptor rule.” Remarkably, this exclusion is observed even among OR-containing transgenes integrated in tandem on the same chromosome (Serizawa et al. 2000). How do rodent OSNs ensure that only a single allele of one OR is functionally expressed? Although vertebrate antigen receptor genes are stochastically chosen by irreversible DNA rearrangements, OR gene choice does not operate by such a mechanism (Eggan et al. 2004; Li et al. 2004). Monogenic OR expression appears to be established by the combination of positive and negative regulation (reviewed in Serizawa et al. 2004). Positive regulation is proposed to operate via a *cis*-acting locus control region (LCR) capable of activating only one OR gene promoter at a time by physical interaction

Comparative Views on Olfactory Axon Guidance

(Serizawa et al. 2003; Lomvardas et al. 2006). Because there are more than 40 different OR gene clusters, each of which may contain its own LCR, the LCR model alone cannot account for monogenic OR expression (Fuss et al. 2007; Nishizumi et al. 2007).

Once a functional OR gene is expressed, the OSN must have a mechanism to prevent the expression of all other ORs, including the allelic OR expressed from the opposite chromosome. There is evidence that the functional OR protein itself contributes to the suppression OR expression from other loci. The deletion of, or a frame shift mutation within, the OR coding region permits the coexpression of a second OR gene (Serizawa et al. 2003; Feinstein et al. 2004; Lewcock and Reed 2004; Shykind et al. 2004). Forced expression of an OR using heterologous promoters can suppress the expression of endogenous ORs (Nguyen et al. 2007; Fleischmann et al. 2008). Because OR-mediated negative feedback does not require G protein signaling (Imai et al. 2006), the exact nature of the negative feedback signal is unclear. In young animals, a small number of OSNs misexpresses multiple ORs, which however are developmentally eliminated in an activity-dependent manner (Tian and Ma 2008).

The apparently stochastic mechanism of OR gene choice in the mouse contrasts with deterministic receptor gene choice in the fly. In *Drosophila*, there are approximately 1200 OSNs distributed across the two major sensory organs, the antenna and maxillary palp (Stocker 1994). These organs are covered with three different types of olfactory sensilla, the basiconic, trichoid, and coeloconic. ORs are expressed in all basiconic and trichoid sensilla and one class of coeloconic sensilla, each of which houses between 1 and 4 OSNs (Stocker 1994). The position of identified sensilla is stereotyped between individual flies (de Bruyne et al. 2001). Because the number of neurons is many orders of magnitude smaller than in the mouse, it has been possible to map in detail which OR is expressed in which OSN, to identify the corresponding sensilla type and position, and to determine which odors activates most fly ORs (de Bruyne et al. 2001; Dobritsa et al. 2003;

T. Imai et al.

Hallem et al. 2004; Couto et al. 2005; Goldman et al. 2005; Hallem and Carlson 2006). From this comprehensive body of work, it is known that the identity of ORs in a given sensillum is tightly regulated (Dobritsa et al. 2003; Hallem et al. 2004; Couto et al. 2005) and depends on Notch signaling (Endo et al. 2007). Members of the POU homeodomain transcription factor family govern the selective expression of ORs in defined OSNs (Clyne et al. 1999a; Ray et al. 2007; Ray et al. 2008). Although *Drosophila* OSNs typically express a single OR together with the Or83b coreceptor, there are numerous instances of two or three ligand-binding ORs coexpressed in a single OSN (Goldman et al. 2005). Further, there is no evidence of either monoallelic expression or the exclusion mechanisms observed in rodents. OSNs in the fly can be transgenically altered to express ectopic ORs, which change the functional tuning of the reprogrammed OSN (Dobritsa et al. 2003; Goldman et al. 2005). Insect OR gene regulation therefore differs in being simpler and more deterministic than the poorly understood process occurring in mammals.

Spatial Organization of the Rodent Olfactory Epithelium and *Drosophila* Antenna

In both rodents and the fly, peripheral olfactory organs show functional organization. In rodents, OSNs expressing a given type of OR are scattered in a restricted area, or zone, in the OE (Ressler et al. 1993; Vassar et al. 1993). Using zone-specific marker genes, one can divide the OE into the dorsomedial (D) and ventrolateral (V) zones (Fig. 1B). The OR genes are divided phylogenetically into two different classes, I and II, based on homology of deduced amino acid sequences (Glusman et al. 2001; Zhang and Firestein 2002; Tsuboi et al. 2006). Although Class I ORs are mostly expressed within the D zone (Zhang et al., 2004; Tsuboi et al., 2006), class II OR genes are expressed in both D and V zones. Within the D zone, class I and class II expressing cells are intermingled and there is no restricted distribution for both classes (Tsuboi et al. 2006). In the V zone, however, each class II OR gene possesses its

unique expression area, distributed in a continuous and overlapping manner along the dorsomedial–ventrolateral axis in the OE (Miyamichi et al. 2005).

In *Drosophila*, sensilla types are distributed across the antenna in a pattern reminiscent of zones in the mouse OE. Trichoid sensilla are restricted to the lateral–distal portion of the antenna, whereas large basiconic sensilla are concentrated in the medial–proximal region of the antenna. Small basiconic and coeloconic sensilla are interspersed in other regions of the antenna. OR gene expression is dispersed among these sensilla types, with a given OR being expressed in ~10–50 OSNs (Clyne et al. 1999b; Vosshall et al. 1999). As in the mouse, OSNs expressing a given OR are interspersed among OSNs expressing different receptors.

OLFACTORY CIRCUITS IN THE BRAIN

Axonal Convergence and the Topographic Map in the Rodent Olfactory Bulb

How is odor information represented in the OB? Each OSN projects a single unbranched axon to the ipsilateral olfactory bulb (OB) of the brain, where it forms synapses with second-order neurons, the mitral and tufted cells. OSNs expressing the same OR converge their axons to two of ~2000 glomeruli located in stereotyped positions on the OB, one on the medial and the other on the lateral surface (Ressler et al. 1994; Vassar et al. 1994; Mombaerts et al. 1996). As a consequence of this topographic map formation, each OR—and the odors that activate it—is represented in the OB as the pattern of activated glomeruli.

In the OB, odorant signals originating in OSNs are relayed to two types of excitatory projection neurons, the mitral and tufted cells. Each glomerulus is innervated by 20–50 mitral/tufted cells, each of which innervates a single glomerulus with a single primary dendrite. Tufted cells project axon branches to the isotypic olfactory columns on the mirror-symmetrical positions, forming intrabulbar connections (Lodovichi et al. 2003). In addition

to the excitatory neurons, there are several types of inhibitory interneurons, the periglomerular cells and granule cells (reviewed in Wilson and Mainen 2006).

Topographic Mapping in the *Drosophila* Antennal Lobe

The antennal lobe in *Drosophila* is anatomically homologous to the rodent olfactory bulb, but significantly simpler because it houses only ~50 glomeruli (Laissue et al. 1999). OSNs in the antenna and maxillary palp each extend a single axon, which fasciculates into the antennal or maxillary nerve and initially targets the periphery of the antennal lobe before individual axons target stereotyped glomeruli (Fig. 2). Within glomeruli, OSNs synapse with projection neurons (PNs), the functional equivalent of mitral and tufted cells. Each PN extends a dendrite that branches within a single glomerulus and an axon that targets higher brain regions, the mushroom body and lateral horn of the protocerebrum. Local inhibitory neurons interact with both OSNs and PNs to modulate olfactory signals (reviewed in Wilson and Mainen 2006).

As in mice, all fly OSNs expressing a given OR target a single glomerulus (Gao et al. 2000; Vosshall et al. 2000; Couto et al. 2005; Fishilevich and Vosshall 2005) (Fig. 2B,C). Unlike in the mouse, there is no obvious relationship between the position of a group of OR-expressing OSNs in the periphery and its target in the antennal lobe (Fig. 2D). However, there is a rough organization of glomeruli according to sensilla type: trichoid OSNs tend to target the anterior–lateral antennal lobe, coeloconic sensilla mostly target the posterior antennal lobe, and basiconic sensilla the anterior medial–dorsal antennal lobe.

MECHANISMS OF OLFACTORY MAP FORMATION IN MAMMALS

Topographic Correlations between the Epithelium and Bulb

Wiring up several millions OSNs expressing up to 1000 ORs to 2000 OB glomeruli is a

Comparative Views on Olfactory Axon Guidance

staggering organizational problem. One parameter that defines the axonal projection site is positional information in the OE. Antibody staining of zone-specific molecules showed a zone-to-zone correlation: OCAM-positive V zone OSNs project axons to the ventral (V) domain of the OB, whereas NQO-1-positive D zone OSNs project axons to the dorsal (D) domain of the OB (Alenius and Böhm 1997; Yoshihara et al. 1997; Yoshihara and Mori 1997; Gussing and Böhm 2004) (Fig. 1B). Systematic *in situ* hybridization and genetic labeling indicated that OSNs expressing class I ORs project their axons to the most anterodorsal areas (DI) within the D domain (Tsuboi et al. 2006; Kobayakawa et al. 2007; Bozza et al. 2009). The OR-specific expression area along the dorsomedial–ventrolateral axis of the V zone OE is well correlated with D–V positioning of glomeruli in the OB (Miyamichi et al. 2005). In contrast, no discernible correlation was found for the anterior–posterior (A–P) axis. These observations suggest that spatial information in the OE contributes to D–V glomerular positioning in the OB. This notion was also supported by transgenic experiments. When the expression areas of ORs were shifted or broadened, projection sites adjusted accordingly along the D–V axis in the OB (Vassalli et al. 2002; Miyamichi et al. 2005).

Neuropilin-2 (Nrp2) and Robo-2 are good candidate guidance molecules determining the D–V axis, because they show graded expression in the OE (Norlin et al. 2001; Cho et al. 2007). Nrp2 is expressed in a ventral-high and dorsal-low gradient, whereas Robo-2 expression shows a dorsal-high and ventral-low gradient in the OE and OSN axon termini (Fig. 1B). Mice deficient for Robo-2 have defective axonal projections along the D–V axis (Cho et al. 2007; Nguyen-Ba-Charvet et al. 2008).

Each OB has mirror-symmetric olfactory maps on the medial (M) and lateral (L) sides. OSNs in the anterior and lateral OE tend to project axons to the lateral OB, whereas OSNs in the medial and posterior OE tend to project axons to the medial OB (Levai et al. 2003). Disruption of IGF signaling affects OSN projections to the lateral OB, although neither

T. Imai et al.

IGF nor IGF receptors show apparent M–L asymmetry (Scolnick et al. 2008). The molecules that distinguish the M–L OB are not known.

OR-Instructed Glomerular Positioning along the A–P Axis

An early and appealing proposal to solve the complex mammalian OSN wiring problem posited that the ORs themselves provided topographic information. The involvement of OR proteins in OSN projections was investigated using genetically engineered mutant mice (Mombaerts et al. 1996). When the coding region of a particular OR gene was replaced with that of another OR gene, axons expressing the swapped OR gene targeted a novel position in the OB (Wang et al. 1998; Feinstein and Mombaerts 2004). Thus, the OR protein appeared to play an instructive role in projecting OSN axons (Mombaerts 2006). Interestingly, OR proteins can be detected not only in olfactory cilia and soma, but also at axon termini (Barnea et al. 2004; Strotmann et al. 2004).

Later experiments complicated the simple view that OR proteins directly instructed guidance decisions. Instead, it appears that OR-instructed axonal projection is regulated by G protein-mediated cAMP signaling independent of the odor ligand specificity of a given OR (Imai et al. 2006; Chesler et al. 2007). Axons expressing a mutant OR-I7 that cannot activate G proteins remained in the anterior region of the OB and failed to converge to a specific glomerulus. However, coexpression of a constitutively active $G_{\alpha s}$ mutant restored axonal convergence and glomerular formation defects. Partial rescue was also observed with the constitutive active mutants of PKA and CREB. Thus, $G_{\alpha s}$ and PKA-mediated transcriptional regulation is required for OSN projections. This conclusion is consistent with earlier findings that $G_{\alpha s}$ -coupled β_2 -adrenergic receptor can instruct OSN projection when swapped with OR-M71, whereas the $G_{\alpha i2}$ -coupled vomeronasal receptor, V1rb2, cannot (Feinstein et al. 2004). Further, constitutively active $G_{\alpha s}$ or dominant-negative PKA, when coexpressed

with wild-type OR-I7, causes a posterior or anterior shift of glomeruli, respectively (Imai et al. 2006). Topographic map formation in the OB is disrupted in ACIII mutant mice (Col et al. 2007; Zou et al. 2007). These findings suggest that the quantitative levels of OR-derived cAMP signals determine A–P positioning of OB glomeruli independent of the function of the OR as ligand-binding receptor (reviewed in Imai and Sakano 2007).

How do different levels of cAMP originating from G-protein signaling lead to differential A–P positioning of OSN axons in the OB? cAMP regulates the transcription of axon guidance molecules such as Neuropilin-1 (Nrp1) (Imai et al. 2006) (Fig. 3). When protein levels are measured in axon termini of OSNs, Nrp1 was found in an anterior-low and posterior-high gradient. Increases or decreases of Nrp1 caused posterior or anterior glomerular shifts, respectively (Imai et al. 2009). Consistent with the hypothesis that Nrp1 regulates A–P positioning, the A–P topography of the glomerular map is perturbed in mice deficient for Nrp1 or its repulsive ligand, Sema3A (Schwartz et al. 2000; Taniguchi et al. 2003; Imai et al. 2009).

Axon–Axon Interactions and Topography of the Olfactory Bulb Map

How do Nrp1 and Sema3A establish the A–P topography of the olfactory map? It is generally believed that the topography of the neural map is determined by positional cues present on the target. However, OSN axons projecting to distinct destinations are presorted in the axon bundle before arrival at the OB, suggesting that pretarget mechanisms also account for the topographic map formation. Indeed, A–P topography of OSN axons can be formed in *Gli3* mutant mice that completely lack the OB (St John et al. 2003; Imai et al. 2009). Gain-of-function and loss-of-function experiments revealed that Nrp1 levels determine the pattern of pretarget axon sorting in the bundles. Nrp1 and its repulsive ligand, Sema3A, are expressed in a complementary manner in OSNs (Fig. 3). OSN-specific knockout of Sema3A altered the

Figure 3. Olfactory sensory neuron projections along the anterior–posterior axis in the mouse olfactory system. Each OR is thought to generate a unique level of cAMP signaling, which is converted to relative expression levels of axon guidance molecules such as *Nrp1* and *Sema3A* via cAMP-dependent protein kinase A (PKA) and cAMP response element binding protein (CREB). An axon guidance receptor, *Nrp1*, and its repulsive ligand, *Sema3A*, are expressed in a complementary manner in OSNs and regulate pretarget sorting of axons. This axon sorting mechanism may be important to establish the topographic order in the OB based on the relative expression levels of guidance molecules expressed by axons. Once OSN axons are sorted, they need to be oriented along the correct axis before projecting onto the OB. This probably requires an intermediate cue, possibly encoded by *Sema3A* derived from the target or along the pathway between the OE and OB. A, anterior; P, posterior.

pretarget axon sorting as well as olfactory map topography (Imai et al. 2009). Thus, pretarget axon–axon interaction is important for topographic map formation (Imai et al. 2009). In the visual system, the “relative” expression levels of axon guidance receptors determine axonal projection sites (Brown et al. 2000). Axon–axon interactions may be a general strategy to pattern topographic maps based on relative expression level of axon guidance molecules expressed by axons.

Theoretically, however, axon–axon interactions alone cannot determine the axis of the map. Guidance cues on the target, as well as intermediary cues, may help orient the pre-sorted axons to a correct orientation on the target. In the olfactory system, *Sema3A* expressed by ensheathing glia appears to function as an intermediate cue. Such intermediate guidance

cues have been reported for the topographic projections of thalamo-cortical axons (Dufour et al. 2003; Seibt et al. 2003).

Axon–axon interactions may be important for the process of OSN regeneration as well (St. John and Key 2003). OSNs turn over continuously throughout life, and newly generated OSNs project axons to the preexisting map with high precision (Gogos et al. 2000). The ability of the olfactory map to rewire itself even in older animals provides a mechanism to maintain constancy in odor perception throughout life.

Neuronal Activity-dependent Local Axon Sorting to Form the Discrete Map

After OSN axons are sorted to approximate D–V and A–P positions in the OB, they are further sorted locally to establish the discrete

T. Imai et al.

glomerular structures and such sorting is influenced by neuronal activity in OSNs. The *CNGA2* gene is X-linked and, therefore, female heterozygous mutant of this gene are mosaic because of random X-inactivation (Zhao and Reed 2001). In these mice, glomeruli for some ORs are duplicated to produce one *CNGA2*-positive and one *CNGA2*-negative glomerulus (Zheng et al. 2000; Serizawa et al. 2006). Genetic silencing of neuronal activity in OSNs perturbs

local fasciculation of OSN axons (Yu et al. 2004). These observations indicate that neuronal activity affects local sorting of OSN axons.

In OSNs, neuronal activity regulates various genes such as those coding for homophilic adhesive molecules Kirrel2/Kirrel3 and repulsive molecules ephrin-A5/EphA5, thereby regulating local axon sorting (Serizawa et al. 2006) (Fig. 4). In *CNGA2* knockout mouse, *Kirrel2* and *EphA5* were down-regulated, whereas

Figure 4. Activity-dependent local axon sorting to form a discrete map on the mouse olfactory bulb. Although OR/cAMP signals regulate coarse targeting of OSN axons along the A–P axis at an earlier stage of patterning, neuronal activity generated by OR/cAMP signals regulates local axon sorting at a later stage. This may indicate that cAMP signals are generated by different mechanisms in earlier and later stages (reviewed in Imai and Sakano 2008). Levels of neuronal activity determine different sets of axon guidance and adhesion molecules, including Kirrel2/Kirrel3, ephrin-A/EphA, and BIG2. Expression levels of these molecules are affected by nares occlusion, although those of *Nrp1/Sema3A* are not. Kirrel2/Kirrel3 mediates local fasciculation of like axons via homophilic adhesion activities, whereas ephrin-A/EphA is thought to segregate heterotypic axons via contact repulsion. BIG2 is involved in heterophilic adhesion. (CNG) cyclic nucleotide gated channel, (ACIII) adenylyl cyclase III.

Kirrel3 and *ephrin-A5* were up-regulated, indicating that these genes are transcribed in an activity-dependent manner. Gain of function of these genes with transgenic mosaic analysis causes duplication of glomeruli for some ORs (Cutforth et al. 2003; Serizawa et al. 2006). In the axon termini of OSNs, these molecules are expressed at differential levels in an OR-specific manner, showing a mosaic pattern of glomeruli with different expression levels. *BIG2* is also expressed at axon termini of OSNs in an OR-specific manner, and facilitates local axon sorting with unknown heterophilic binding partners (Kaneko-Goto et al. 2008).

Activity-Dependent Refinement and Maintenance of the Olfactory Bulb Map

Activity-dependent refinement, which follows the initial targeting processes, plays an important role in many sensory systems during development. In the mouse olfactory system, satellite glomeruli are ectopically formed in young animals. Such glomeruli are gradually eliminated with age, refining the glomerular map. In mice deficient for *CNGA2* or whose nares are surgically occluded, these ectopic glomeruli persist longer or fail to be eliminated altogether (Zheng et al. 2000; Nakatani et al. 2003; Zou et al. 2004).

Neuronal activity is required for the maintenance of the glomerular map (Zhao and Reed 2001). In mosaic female mice, *CNGA2*-negative cells are eliminated when they face competition from neighboring functional OSNs, but survive under the noncompetitive conditions operating under nares occlusion. Thus refinement and maintenance appears to be regulated in part at the level of cell survival in the mouse.

MECHANISMS OF OLFACTORY MAP FORMATION IN *DROSOPHILA*

Same but Different: Divergent Mechanisms of Glomerular Targeting in Flies

Convergence of like axons to stereotyped glomeruli where synapses form with relay and local interneurons is a feature of both mammalian

Comparative Views on Olfactory Axon Guidance

and insect olfactory systems. However, the underlying developmental mechanisms are remarkably different. First, there is a considerable difference in scale between mouse and fly olfactory systems, with the fly having ~4000-fold fewer OSNs, ~40-fold fewer glomeruli, and ~20-fold fewer ORs. Second, fly OSNs do not regenerate throughout the life of the animal. Antennal lobe connections formed during development are fixed throughout life. Third, ORs appear to play no functional role in wiring the glomerular map. Flies lacking the *Or83b* coreceptor, required for basal spontaneous and odor-evoked activity in OSNs, show normal map formation (Larsson et al. 2004). OSNs retaining *Or83b* but lacking the ligand-binding receptor *Or22a* target the appropriate *DM2* glomerulus (Fig. 2B,C), and this is not altered by ectopic expression of another receptor, *Or47a* (Dobritsa et al. 2003). Further, there is also no evidence of OR protein localized to OSN axons (Elmore and Smith 2001; Dobritsa et al. 2003; Larsson et al. 2004). Fourth, there is no evidence for monogenic or monoallelic expression. All fly OSNs express at least two ORs, one or more ligand-binding ORs and the *Or83b* coreceptor, and there is no regulatory barrier preventing the transgenic expression of additional ORs in a given OSN. Fifth, there is no evidence of a requirement for neuronal activity for the establishment, refinement, or maintenance of the glomerular map. The antennal lobe map is remarkably stable even when all odor-evoked activity is eliminated (Larsson et al. 2004) or when populations of target PNs or afferent OSNs are ablated (Tanaka et al. 2004; Berdnik et al. 2006). Therefore the insect antennal lobe is patterned by hard-wired, OR- and activity-independent mechanisms distinct from those used by mammals.

Independent Developmental Specification of Input and Output Neurons

What are the rules that wire up the insect olfactory system, if the ORs themselves have no influence over map formation? The smaller scale of the *Drosophila* olfactory system, and the availability of excellent cell-type specific markers

T. Imai et al.

such as the ORs, has made this a powerful system to study the rules of axon guidance. The ~50 *Drosophila* glomeruli have stereotyped shapes, sizes, and positions in the antennal lobe (Laissue et al. 1999) and forward genetics can be used to identify genes that disrupt any aspect of the wiring problem: The specification of OSN identity, the precise targeting of OSN axons, and the dendritic patterning of PNs.

An important early discovery in this field was that OSNs and PNs are independently specified to target a defined area in the antennal lobe. Asymmetric Notch signaling is important to specify the identity of OSNs in a given sensillum (Endo et al. 2007) (Fig. 5A). Notch signaling in OSN precursors initiates a series of lineage decisions that directs both the choice of an OR and glomerular target in the antennal lobe. Combinatorials of POU homeodomain BTB-zinc finger transcription factors govern both OR gene choice and wiring specificity of OSNs and PNs (Clyne et al. 1999a; Komiyama et al. 2003; Komiyama et al. 2004; Zhu et al. 2006b; Spletter et al. 2007). Cells that are physically adjacent in the antenna can target either adjacent glomeruli or widely separated glomeruli in the antennal lobe. Therefore, the spatial relationships that govern position in the rodent OE and targets in the OB are not evident in the fly.

For OSN axons to synapse appropriately with PN dendrites, two contrasting models can be envisioned: Either afferent or target neurons could set up a prepattern that then attracts the matching neuron or OSNs and PNs could be independently specified to target the appropriate region. To test these models, Luo and colleagues used the powerful MARCM (mosaic analysis with repressible cell marker) technique (Lee and Luo 1999) to label individual PN precursors during fly development. They discovered that PNs are prespecified by birth order and cell lineage to target a specific glomerulus and do so before the arrival of OSNs (Jefferis et al. 2001) (Fig. 5B). Careful analysis of the developmental timing of OSN axon extension further suggested that PNs establish a glomerular prepattern long before the arrival of incoming OSNs (Jefferis et al. 2004). This suggests that

global patterning cues exist in the antennal lobe to permit a rough spatial patterning of PN dendrites that is later refined both by interactions between PNs and also the later influence of incoming OSN axons. One of these cues is Wnt5 signaling mediated by the derailed receptors, Drl and Drl-2. When these signaling pathways are disrupted, both OSN axons and PN dendrites are disorganized (Yao et al. 2007; Sakurai et al. 2009).

Mechanisms of Olfactory Axon Guidance and Dendritic Patterning in the *Drosophila* Antennal Lobe

Forward genetic screens in *Drosophila* have identified a number of cell adhesion and axon guidance molecules that instruct axon and dendrite patterning in the antennal lobe (Fig. 5B). Mutant animals in which all OSNs lack N-cadherin show anomalies in glomerular map formation. OSN axons arrest at the border of the antennal lobe and fail to invade their appropriate glomerular targets, and accordingly glomeruli fail to form in N-cadherin mutants (Hummel and Zipursky 2004). When subsets of N-cadherin-negative OSNs were examined, their axons were seen to target an area of the external surface of the bulb that corresponds roughly with the wild type glomerular position (Hummel and Zipursky 2004). Therefore, OSNs are likely to be specified to recognize global cues, independent of positional information encoded by PN dendrites. N-cadherin also plays an important role in patterning PN dendrites. PNs lacking N-cadherin target their target glomeruli appropriately, but show ectopic innervation of adjacent glomeruli (Zhu and Luo 2004). Therefore dendrite–dendrite interactions mediated by N-cadherin serve to restrict PN dendrites to a single glomerulus.

What are the mechanisms that restrict OSN and PN processes to a single glomerulus? Some clues come from analysis of flies mutant for the Ig-superfamily protein DSCAM, which uses alternative splicing to generate up to 38,000 distinct protein isoforms. The axonal targeting of a subset of OSNs requires DSCAM (Hummel et al. 2003; Hattori et al. 2007) and DSCAM

Comparative Views on Olfactory Axon Guidance

Figure 5. Molecular determinants of neuronal identity and glomerular targeting in the *Drosophila* antennal lobe. (A), Notch signaling specifies cell fate of OSNs housed in the same sensillum. Three arbitrary sensilla (1–3) housing either 2 or 3 OSNs are depicted with the corresponding axonal targets of the individual OSNs. Although Notch^{ON} and Notch^{OFF} cells are adjacent in a given antennal sensillum, their targets are broadly dispersed in the antennal lobe (Endo et al. 2007). (B), Schematic of developmental events and pathways patterning the fly olfactory system. In early pupal stages PNs have already targeted dendrites to appropriate positions where future glomeruli will form, whereas OSN axons have only reached the edge of the antennal lobe. In midpupation, some OSN axons begin to enter the antennal lobe and target the appropriate glomerular region. Late in pupal life, all OSNs have arrived and synaptic matching refines the connections of OSN axons and PN dendrites. The genes known to be involved in each of these steps are indicated and discussed in the text.

mutant PNs fail to elaborate fully branched dendrites (Zhu et al. 2006a). When DSCAM is overexpressed in a subset of PNs, their dendrites were shifted ventrally to a novel glomerulus and the OSNs that normally synapse with the PNs were correspondingly shifted to the new position (Zhu et al. 2006a). When the diversity of

DSCAM isoforms was artificially reduced to a single protein, OSN axons were severely disordered and formed many ectopic connections (Hattori et al. 2007). This suggests that DSCAM organizes axonal projections of OSNs and coordinates matching between OSN axons and PN dendrites. It remains to be resolved how and

T. Imai et al.

whether the diversity of DSCAM isoforms instructs glomerular specificity of either or both cell types, although an instructional role for DSCAM has been shown at other fly synapses (Hattori et al. 2009).

Gradients of signaling and axon guidance molecules are crucial for the correct patterning of the mammalian OB. Although *Sema3A/Nrp1* controls A–P glomerular targeting of mouse OSNs, the *Drosophila* semaphorin *Sema-1a* plays a role in the patterning of both OSN axons and PN dendrites (Komiya et al. 2007; Lattemann et al. 2007; Sweeney et al. 2007). In OSNs, *Sema-1a*/PlexinA-mediated axon–axon interaction is required to sort early-arriving and late-arriving axons and prevent mistargeting (Lattemann et al. 2007; Sweeney et al. 2007). In PN dendrites, *Sema-1a* is expressed in a D–L high to V–M low gradient that is important for proper dendritic targeting in a cell-autonomous fashion (Komiya et al. 2007). Although *Sema-1a* shows continuous and graded expression in PN dendrites, recent work suggests a new mechanism for discrete patterning of PN dendrites. The transmembrane leucine-rich repeat proteins *Capricious* (Caps) and *Tartan* (Trn) were found to be expressed in subsets of PNs that target distinct glomeruli (Hong et al. 2009). When *caps* is deleted from *Caps*⁺ PNs, their dendrites invade glomeruli occupied by *Caps*[−] PNs. Thus Caps provides a mechanism to assign a discrete cell–surface identity code to different PNs. Because Caps provides information to divide the antennal lobe into two populations, additional cell–surface codes still to be described would be required to specify a unique identity to each of the ~50 antennal lobe glomeruli.

CONCLUDING REMARKS

In both mammals and insects, olfactory information is organized according to the selective wiring of axons linked to OSNs expressing the same OR. This elegant organizational scheme arrays olfactory information in a receptor-topic fashion on the olfactory bulb and antennal lobe. In both experimental systems, axon–axon interactions play an important role as do

gradients of axon guidance molecules that provide global positional information. Although the final pattern of the primary olfactory center is homologous in rodents and flies, the detailed molecular mechanisms that give rise to this pattern are largely distinct. This suggests mechanisms of convergent evolution acted to produce an optimal solution to wiring olfactory information into the brain. There has been great progress toward elucidating how large numbers of molecularly distinct OSN axons target distinct glomeruli in the brain. What remains to be solved is the exact molecular code that matches OSNs and postsynaptic neurons to unique glomeruli. Once solved, such a code would likely provide insights into synaptic patterning throughout the nervous system.

ACKNOWLEDGMENTS

T.I. is supported by the JST PRESTO program. H.S. is supported by a Specially Promoted Research Grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan. L.B.V. is supported by grants from the National Institutes of Health and by an Investigator Award from the Howard Hughes Medical Institute.

REFERENCES

- Alenius M, Bohm S. 1997. Identification of a novel neural cell adhesion molecule-related gene with a potential role in selective axonal projection. *J Biol Chem* **272**: 26083–26086.
- Axel R. 1995. The molecular logic of smell. *Sci Am* **273**: 154–159.
- Barnea G, O'Donnell S, Mancina F, Sun X, Nemes A, Mendelsohn M, Axel R. 2004. Odorant receptors on axon termini in the brain. *Science* **304**: 1468.
- Belluscio L, Gold GH, Nemes A, Axel R. 1998. Mice deficient in *G(olf)* are anosmic. *Neuron* **20**: 69–81.
- Benton R, Sachse S, Michnick SW, Vosshall LB. 2006. Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors *in vivo*. *PLoS Biol* **4**: e20.
- Berdnik D, Chihara T, Couto A, Luo L. 2006. Wiring stability of the adult *Drosophila* olfactory circuit after lesion. *J Neurosci* **26**: 3367–3376.
- Bozza T, Vassalli A, Fuss S, Zhang JJ, Weiland B, Pacifico R, Feinstein P, Mombaerts P. 2009. Mapping of class I and class II odorant receptors to glomerular domains by two distinct types of olfactory sensory neurons in the mouse. *Neuron* **61**: 220–233.

- Brennan PA, Zufall F. 2006. Pheromonal communication in vertebrates. *Nature* **444**: 308–315.
- Brown A, Yates PA, Burrola P, Ortuno D, Vaidya A, Jessell TM, Pfaff SL, O'Leary DD, Lemke G. 2000. Topographic mapping from the retina to the midbrain is controlled by relative but not absolute levels of EphA receptor signaling. *Cell* **102**: 77–88.
- Brunet LJ, Gold GH, Ngai J. 1996. General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. *Neuron* **17**: 681–693.
- Buck L, Axel R. 1991. A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell* **65**: 175–187.
- Chesler AT, Zou DJ, Le Pichon CE, Peterlin ZA, Matthews GA, Pei X, Miller MC, Firestein S. 2007. A G protein/cAMP signal cascade is required for axonal convergence into olfactory glomeruli. *Proc Natl Acad Sci U S A*.
- Chess A, Simon I, Cedar H, Axel R. 1994. Allelic inactivation regulates olfactory receptor gene expression. *Cell* **78**: 823–834.
- Cho JH, Lepine M, Andrews W, Parnavelas J, Cloutier JE. 2007. Requirement for Slit-1 and Robo-2 in zonal segregation of olfactory sensory neuron axons in the main olfactory bulb. *J Neurosci* **27**: 9094–9104.
- Clyne PJ, Certel SJ, de Bruyne M, Zaslavsky L, Johnson WA, Carlson JR. 1999a. The odor specificities of a subset of olfactory receptor neurons are governed by *Acj6*, a POU-domain transcription factor. *Neuron* **22**: 339–347.
- Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR. 1999b. A novel family of divergent seven-transmembrane proteins: Candidate odorant receptors in *Drosophila*. *Neuron* **22**: 327–338.
- Col JA, Matsuo T, Storm DR, Rodriguez I. 2007. Adenylyl cyclase-dependent axonal targeting in the olfactory system. *Development* **134**: 2481–2489.
- Couto A, Alenius M, Dickson BJ. 2005. Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. *Curr Biol* **15**: 1535–1547.
- Cutforth T, Moring L, Mendelsohn M, Nemes A, Shah NM, Kim MM, Frisen J, Axel R. 2003. Axonal ephrin-As and odorant receptors: Coordinate determination of the olfactory sensory map. *Cell* **114**: 311–322.
- de Bruyne M, Foster K, Carlson JR. 2001. Odor coding in the *Drosophila* antenna. *Neuron* **30**: 537–552.
- Dobritsa AA, van der Goes van Naters W, Warr CG, Steinbrecht RA, Carlson JR. 2003. Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. *Neuron* **37**: 827–841.
- Dufour A, Seibt J, Passante L, Depaepe V, Ciossek T, Frisen J, Kullander K, Flanagan JG, Polleux F, Vanderhaeghen P. 2003. Area specificity and topography of thalamocortical projections are controlled by ephrin/Eph genes. *Neuron* **39**: 453–465.
- Eggan K, Baldwin K, Tackett M, Osborne J, Gogos J, Chess A, Axel R, Jaenisch R. 2004. Mice cloned from olfactory sensory neurons. *Nature* **428**: 44–49.
- Elmore T, Smith DP. 2001. Putative *Drosophila* odor receptor OR43b localizes to dendrites of olfactory neurons. *Insect Biochem Mol Biol* **31**: 791–798.
- Endo K, Aoki T, Yoda Y, Kimura K, Hama C. 2007. *Notch* signal organizes the *Drosophila* olfactory circuitry by diversifying the sensory neuronal lineages. *Nat Neurosci* **10**: 153–160.
- Engsontia P, Sanderson AP, Cobb M, Walden KK, Robertson HM, Brown S. 2008. The red flour beetle's large nose: An expanded odorant receptor gene family in *Tribolium castaneum*. *Insect Biochem Mol Biol* **38**: 387–397.
- Feinstein P, Mombaerts P. 2004. A contextual model for axonal sorting into glomeruli in the mouse olfactory system. *Cell* **117**: 817–831.
- Feinstein P, Bozza T, Rodriguez I, Vassalli A, Mombaerts P. 2004. Axon guidance of mouse olfactory sensory neurons by odorant receptors and the beta2 adrenergic receptor. *Cell* **117**: 833–846.
- Fishilevich E, Vosshall LB. 2005. Genetic and functional subdivision of the *Drosophila* antennal lobe. *Curr Biol* **15**: 1548–1553.
- Fleischmann A, Shykind BM, Sosulski DL, Franks KM, Glinka ME, Mei DF, Sun Y, Kirkland J, Mendelsohn M, Albers MW et al. 2008. Mice with a "monoclonal nose": Perturbations in an olfactory map impair odor discrimination. *Neuron* **60**: 1068–1081.
- Fuss SH, Omura M, Mombaerts P. 2007. Local and cis effects of the H element on expression of odorant receptor genes in mouse. *Cell* **130**: 373–384.
- Gao Q, Yuan B, Chess A. 2000. Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. *Nat Neurosci* **3**: 780–785.
- Gilbert AN. 2008. *What the nose knows: The science of scent in everyday life*. Crown, New York.
- Glusman G, Yanai I, Rubin I, Lancet D. 2001. The complete human olfactory subgenome. *Genome Res* **11**: 685–702.
- Gogos JA, Osborne J, Nemes A, Mendelsohn M, Axel R. 2000. Genetic ablation and restoration of the olfactory topographic map. *Cell* **103**: 609–620.
- Goldman AL, Van der Goes van Naters W, Lessing D, Warr CG, Carlson JR. 2005. Coexpression of two functional odor receptors in one neuron. *Neuron* **45**: 661–666.
- Gussing F, Böhm S. 2004. NQO1 activity in the main and the accessory olfactory systems correlates with the zonal topography of projection maps. *Eur J Neurosci* **19**: 2511–2518.
- Hallem EA, Carlson JR. 2006. Coding of odors by a receptor repertoire. *Cell* **125**: 143–160.
- Hallem EA, Ho MG, Carlson JR. 2004. The molecular basis of odor coding in the *Drosophila* antenna. *Cell* **117**: 965–979.
- Hattori D, Chen Y, Matthews BJ, Salwinski L, Sabatti C, Grueber WB, Zipursky SL. 2009. Robust discrimination between self and non-self neurites requires thousands of Dscam1 isoforms. *Nature* **461**: 644–648.
- Hattori D, Demir E, Kim HW, Viragh E, Zipursky SL, Dickson BJ. 2007. Dscam diversity is essential for neuronal wiring and self-recognition. *Nature* **449**: 223–227.
- Hildebrand JG, Shepherd GM. 1997. Mechanisms of olfactory discrimination: Converging evidence for common principles across phyla. *Annu Rev Neurosci* **20**: 595–631.
- Hong W, Zhu H, Potter CJ, Barsh G, Kurusu M, Zinn K, Luo L. 2009. Leucine-rich repeat transmembrane proteins instruct discrete dendrite targeting in an olfactory map. *Nat Neurosci* **12**: 1542–1550.

T. Imai et al.

- Hummel T, Zipursky SL. 2004. Afferent induction of olfactory glomeruli requires N-cadherin. *Neuron* **42**: 77–88.
- Hummel T, Vasconcelos ML, Clemens JC, Fishilevich Y, Vosshall LB, Zipursky SL. 2003. Axonal targeting of olfactory receptor neurons in *Drosophila* is controlled by *Dscam*. *Neuron* **37**: 221–231.
- Imai T, Sakano H. 2007. Roles of odorant receptors in projecting axons in the mouse olfactory system. *Curr Opin Neurobiol* **17**: 507–515.
- Imai T, Sakano H. 2008. Odorant receptor-mediated signaling in the mouse. *Curr Opin Neurobiol* **18**: 251–260.
- Imai T, Suzuki M, Sakano H. 2006. Odorant receptor-derived cAMP signals direct axonal targeting. *Science* **314**: 657–661.
- Imai T, Yamazaki T, Kobayakawa R, Kobayakawa K, Abe T, Suzuki M, Sakano H. 2009. Pre-target axon sorting establishes the neural map topography. *Science* **325**: 585–590.
- Jefferis GS, Vyas RM, Berdnik D, Ramaekers A, Stocker RF, Tanaka NK, Ito K, Luo L. 2004. Developmental origin of wiring specificity in the olfactory system of *Drosophila*. *Development* **131**: 117–130.
- Jefferis GSXE, Marin EC, Stocker RF, Luo L. 2001. Target neuron prespecification in the olfactory map of *Drosophila*. *Nature* **414**: 204–208.
- Jones WD, Nguyen TA, Kloss B, Lee KJ, Vosshall LB. 2005. Functional conservation of an insect odorant receptor gene across 250 million years of evolution. *Curr Biol* **15**: R119–R121.
- Kaneko-Goto T, Yoshihara S, Miyazaki H, Yoshihara Y. 2008. BIG-2 mediates olfactory axon convergence to target glomeruli. *Neuron* **57**: 834–846.
- Kobayakawa K, Kobayakawa R, Matsumoto H, Oka Y, Imai T, Ikawa M, Okabe M, Ikeda T, Itohara S, Kikusui T et al. 2007. Innate versus learned odour processing in the mouse olfactory bulb. *Nature* **450**: 503–508.
- Komiyama T, Carlson JR, Luo L. 2004. Olfactory receptor neuron axon targeting: Intrinsic transcriptional control and hierarchical interactions. *Nat Neurosci* **7**: 819–825.
- Komiyama T, Johnson WA, Luo L, Jefferis GS. 2003. From lineage to wiring specificity. POU domain transcription factors control precise connections of *Drosophila* olfactory projection neurons. *Cell* **112**: 157–167.
- Komiyama T, Sweeney LB, Schuldiner O, Garcia KC, Luo L. 2007. Graded expression of semaphorin-1a cell-autonomously directs dendritic targeting of olfactory projection neurons. *Cell* **128**: 399–410.
- Krieger J, Klink O, Mohl C, Raming K, Breer H. 2003. A candidate olfactory receptor subtype highly conserved across different insect orders. *J Comp Physiol A* **189**: 519–526.
- Laissue PP, Reiter C, Hiesinger PR, Halter S, Fischbach KF, Stocker RF. 1999. Three-dimensional reconstruction of the antennal lobe in *Drosophila melanogaster*. *J Comp Neurol* **405**: 543–552.
- Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H, Vosshall LB. 2004. *Or83b* encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. *Neuron* **43**: 703–714.
- Lattemann M, Zierau A, Schulte C, Seidl S, Kuhlmann B, Hummel T. 2007. Semaphorin-1a controls receptor neuron-specific axonal convergence in the primary olfactory center of *Drosophila*. *Neuron* **53**: 169–184.
- Lee T, Luo L. 1999. Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. *Neuron* **22**: 451–461.
- Levai O, Breer H, Strotmann J. 2003. Subzonal organization of olfactory sensory neurons projecting to distinct glomeruli within the mouse olfactory bulb. *J Comp Neurol* **458**: 209–220.
- Lewcock JW, Reed RR. 2004. A feedback mechanism regulates monoallelic odorant receptor expression. *Proc Natl Acad Sci* **101**: 1069–1074.
- Li J, Ishii T, Feinstein P, Mombaerts P. 2004. Odorant receptor gene choice is reset by nuclear transfer from mouse olfactory sensory neurons. *Nature* **428**: 393–399.
- Lodovichi C, Belluscio L, Katz LC. 2003. Functional topography of connections linking mirror-symmetric maps in the mouse olfactory bulb. *Neuron* **38**: 265–276.
- Lomvardas S, Barnea G, Pisapia DJ, Mendelsohn M, Kirkland J, Axel R. 2006. Interchromosomal interactions and olfactory receptor choice. *Cell* **126**: 403–413.
- Luo L, Flanagan JG. 2007. Development of continuous and discrete neural maps. *Neuron* **56**: 284–300.
- Ma M. 2007. Encoding olfactory signals via multiple chemosensory systems. *Crit Rev Biochem Mol Biol* **42**: 463–480.
- Malnic B, Hirono J, Sato T, Buck LB. 1999. Combinatorial receptor codes for odors. *Cell* **96**: 713–723.
- Miyamichi K, Serizawa S, Kimura HM, Sakano H. 2005. Continuous and overlapping expression domains of odorant receptor genes in the olfactory epithelium determine the dorsal/ventral positioning of glomeruli in the olfactory bulb. *J Neurosci* **25**: 3586–3592.
- Mombaerts P. 2006. Axonal wiring in the mouse olfactory system. *Annu Rev Cell Dev Biol* **22**: 713–737.
- Mombaerts P, Wang F, Dulac C, Chao SK, Nemes A, Mendelsohn M, Edmondson J, Axel R. 1996. Visualizing an olfactory sensory map. *Cell* **87**: 675–686.
- Mori K, Takahashi YK, Igarashi KM, Yamaguchi M. 2006. Maps of odorant molecular features in the mammalian olfactory bulb. *Physiol Rev* **86**: 409–433.
- Nakagawa T, Vosshall LB. 2009. Controversy and consensus: Noncanonical signaling mechanisms in the insect olfactory system. *Curr Opin Neurobiol* **19**: 284–292.
- Nakagawa T, Sakurai T, Nishioka T, Touhara K. 2005. Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. *Science* **307**: 1638–1642.
- Nakatani H, Serizawa S, Nakajima M, Imai T, Sakano H. 2003. Developmental elimination of ectopic projection sites for the transgenic OR gene that has lost zone specificity in the olfactory epithelium. *Eur J Neurosci* **18**: 2425–2432.
- Neuhaus EM, Gisselmann G, Zhang W, Dooley R, Stortkuhl K, Hatt H. 2005. Odorant receptor heterodimerization in the olfactory system of *Drosophila melanogaster*. *Nat Neurosci* **8**: 15–17.
- Nguyen-Ba-Charvet KT, Di Meglio T, Fouquet C, Chedotal A. 2008. Robos and slits control the pathfinding and targeting of mouse olfactory sensory axons. *J Neurosci* **28**: 4244–4249.
- Nguyen MQ, Zhou Z, Marks CA, Ryba NJ, Belluscio L. 2007. Prominent roles for odorant receptor coding sequences in allelic exclusion. *Cell* **131**: 1009–1017.

- Nishizumi H, Kumasaka K, Inoue N, Nakashima A, Sakano H. 2007. Deletion of the core-H region in mice abolishes the expression of three proximal odorant receptor genes in cis. *Proc Natl Acad Sci* **104**: 20067–20072.
- Norlin EM, Alenius M, Gussing F, Hagglund M, Vedin V, Bohm S. 2001. Evidence for gradients of gene expression correlating with zonal topography of the olfactory sensory map. *Mol Cell Neurosci* **18**: 283–295.
- Pitts RJ, Fox AN, Zwiebel LJ. 2004. A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector *Anopheles gambiae*. *Proc Natl Acad Sci* **101**: 5058–5063.
- Ray A, van der Goes van Naters W, Carlson JR. 2008. A regulatory code for neuron-specific odor receptor expression. *PLoS Biol* **6**: e125.
- Ray A, van Naters WG, Shiraiwa T, Carlson JR. 2007. Mechanisms of odor receptor gene choice in *Drosophila*. *Neuron* **53**: 353–369.
- Ressler KJ, Sullivan SL, Buck LB. 1993. A zonal organization of odorant receptor gene expression in the olfactory epithelium. *Cell* **73**: 597–609.
- Ressler KJ, Sullivan SL, Buck LB. 1994. Information coding in the olfactory system: Evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell* **79**: 1245–1255.
- Robertson HM, Warr CG, Carlson JR. 2003. Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* **100**: 14537–14542.
- Sakurai M, Aoki T, Yoshikawa S, Santschi LA, Saito H, Endo K, Ishikawa K, Kimura K, Ito K, Thomas JB et al. 2009. Differentially expressed Drl and Drl-2 play opposing roles in Wnt5 signaling during *Drosophila* olfactory system development. *J Neurosci* **29**: 4972–4980.
- Sato K, Pellegrino M, Nakagawa T, Nakagawa T, Vossall LB, Touhara K. 2008. Insect olfactory receptors are heteromeric ligand-gated ion channels. *Nature* **452**: 1002–1006.
- Schwartz GA, Kostek C, Ahmad N, Dibble C, Pays L, Puschel AW. 2000. Semaphorin 3A is required for guidance of olfactory axons in mice. *J Neurosci* **20**: 7691–7697.
- Scolnick JA, Cui K, Duggan CD, Xuan S, Yuan XB, Efstratiadis A, Ngai J. 2008. Role of IGF signaling in olfactory sensory map formation and axon guidance. *Neuron* **57**: 847–857.
- Seibt J, Schuurmans C, Gradwohl G, Dehay C, Vanderhaeghen P, Guillemot F, Polleux F. 2003. Neurogenin2 specifies the connectivity of thalamic neurons by controlling axon responsiveness to intermediate target cues. *Neuron* **39**: 439–452.
- Serizawa S, Miyamichi K, Sakano H. 2004. One neuron-one receptor rule in the mouse olfactory system. *Trends Genet* **20**: 648–653.
- Serizawa S, Ishii T, Nakatani H, Tsuboi A, Nagawa F, Asano M, Sudo K, Sakagami J, Sakano H, Ijiri T et al. 2000. Mutually exclusive expression of odorant receptor transgenes. *Nat Neurosci* **3**: 687–693.
- Serizawa S, Miyamichi K, Nakatani H, Suzuki M, Saito M, Yoshihara Y, Sakano H. 2003. Negative feedback regulation ensures the one receptor-one olfactory neuron rule in mouse. *Science* **302**: 2088–2094.
- Serizawa S, Miyamichi K, Takeuchi H, Yamagishi Y, Suzuki M, Sakano H. 2006. A neuronal identity code for the odorant receptor-specific and activity-dependent axon sorting. *Cell* **127**: 1057–1069.
- Shykind BM, Rohani SC, O'Donnell S, Nemes A, Mendelsohn M, Sun Y, Axel R, Barnea G. 2004. Gene switching and the stability of odorant receptor gene choice. *Cell* **117**: 801–815.
- Spletter ML, Liu J, Liu J, Su H, Giniger E, Komiyama T, Quake S, Luo L. 2007. *Lola* regulates *Drosophila* olfactory projection neuron identity and targeting specificity. *Neural Dev* **2**: 14.
- St John JA, Clarris HJ, McKeown S, Royal S, Key B. 2003. Sorting and convergence of primary olfactory axons are independent of the olfactory bulb. *J Comp Neurol* **464**: 131–140.
- St John JA, Key B. 2003. Axon mis-targeting in the olfactory bulb during regeneration of olfactory neuroepithelium. *Chem Senses* **28**: 773–779.
- Stephan AB, Shum EY, Hirsh S, Cygnar KD, Reisert J, Zhao H. 2009. ANO2 is the ciliary calcium-activated chloride channel that may mediate olfactory amplification. *Proc Natl Acad Sci U S A* **106**: 11776–11781.
- Stocker RF. 1994. The organization of the chemosensory system in *Drosophila melanogaster*: A review. *Cell Tissue Res* **275**: 3–26.
- Strotmann J, Levai O, Fleischer J, Schwarzenbacher K, Breer H. 2004. Olfactory receptor proteins in axonal processes of chemosensory neurons. *J Neurosci* **24**: 7754–7761.
- Sweeney LB, Couto A, Chou YH, Berdnik D, Dickson BJ, Luo L, Komiyama T. 2007. Temporal target restriction of olfactory receptor neurons by Semaphorin-1a/PlexinA-mediated axon-axon interactions. *Neuron* **53**: 185–200.
- Tanaka NK, Awasaki T, Shimada T, Ito K. 2004. Integration of chemosensory pathways in the *Drosophila* second-order olfactory centers. *Curr Biol* **14**: 449–457.
- Taniguchi M, Nagao H, Takahashi YK, Yamaguchi M, Mitsui S, Yagi T, Mori K, Shimizu T. 2003. Distorted odor maps in the olfactory bulb of semaphorin 3A-deficient mice. *J Neurosci* **23**: 1390–1397.
- Tian H, Ma M. 2008. Activity plays a role in eliminating olfactory sensory neurons expressing multiple odorant receptors in the mouse septal organ. *Mol Cell Neurosci* **38**: 484–488.
- Touhara K, Vossall LB. 2009. Sensing odorants and pheromones with chemosensory receptors. *Annu Rev Physiol* **71**: 307–332.
- Tsuboi A, Miyazaki T, Imai T, Sakano H. 2006. Olfactory sensory neurons expressing class I odorant receptors converge their axons on an antero-dorsal domain of the olfactory bulb in the mouse. *Eur J Neurosci* **23**: 1436–1444.
- Vassalli A, Rothman A, Feinstein P, Zapotocky M, Mombaerts P. 2002. Minigenes impart odorant receptor-specific axon guidance in the olfactory bulb. *Neuron* **35**: 681–696.

T. Imai et al.

- Vassar R, Ngai J, Axel R. 1993. Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. *Cell* **74**: 309–318.
- Vassar R, Chao SK, Sitcheran R, Nunez JM, Vossall LB, Axel R. 1994. Topographic organization of sensory projections to the olfactory bulb. *Cell* **79**: 981–991.
- Vossall LB, Wong AM, Axel R. 2000. An olfactory sensory map in the fly brain. *Cell* **102**: 147–159.
- Vossall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R. 1999. A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96**: 725–736.
- Wang F, Nemes A, Mendelsohn M, Axel R. 1998. Odorant receptors govern the formation of a precise topographic map. *Cell* **93**: 47–60.
- Wicher D, Schafer R, Bauernfeind R, Stensmyr MC, Heller R, Heinemann SH, Hansson BS. 2008. *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. *Nature* **452**: 1007–1011.
- Wilson RI, Mainen ZF. 2006. Early events in olfactory processing. *Annu Rev Neurosci* **29**: 163–201.
- Wistrand M, Kall L, Sonnhämmer EL. 2006. A general model of G protein-coupled receptor sequences and its application to detect remote homologs. *Protein Sci* **15**: 509–521.
- Wong ST, Trinh K, Hacker B, Chan GC, Lowe G, Gaggari A, Xia Z, Gold GH, Storm DR. 2000. Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. *Neuron* **27**: 487–497.
- Yao Y, Wu Y, Yin C, Ozawa R, Aigaki T, Wouda RR, Noordermeer JN, Fradkin LG, Hing H. 2007. Antagonistic roles of Wnt5 and the Drl receptor in patterning the *Drosophila* antennal lobe. *Nat Neurosci* **10**: 1423–1432.
- Yoshihara Y, Mori K. 1997. Basic principles and molecular mechanisms of olfactory axon pathfinding. *Cell Tissue Res* **290**: 457–463.
- Yoshihara Y, Kawasaki M, Tamada A, Fujita H, Hayashi H, Kagamiyama H, Mori K. 1997. OCAM: A new member of the neural cell adhesion molecule family related to zone-to-zone projection of olfactory and vomeronasal axons. *J Neurosci* **17**: 5830–5842.
- Yu CR, Power J, Barnea G, O'Donnell S, Brown HE, Osborne J, Axel R, Gogos JA. 2004. Spontaneous neural activity is required for the establishment and maintenance of the olfactory sensory map. *Neuron* **42**: 553–566.
- Zhang X, Firestein S. 2002. The olfactory receptor gene superfamily of the mouse. *Nat Neurosci* **5**: 124–133.
- Zhang X, Firestein S. 2007. Comparative genomics of odorant and pheromone receptor genes in rodents. *Genomics* **89**: 441–450.
- Zhao H, Reed RR. 2001. X inactivation of the OCNC1 channel gene reveals a role for activity-dependent competition in the olfactory system. *Cell* **104**: 651–660.
- Zheng C, Feinstein P, Bozza T, Rodriguez I, Mombaerts P. 2000. Peripheral olfactory projections are differentially affected in mice deficient in a cyclic nucleotide-gated channel subunit. *Neuron* **26**: 81–91.
- Zhu H, Luo L. 2004. Diverse functions of N-cadherin in dendritic and axonal terminal arborization of olfactory projection neurons. *Neuron* **42**: 63–75.
- Zhu H, Hummel T, Clemens JC, Berdnik D, Zipursky SL, Luo L. 2006a. Dendritic patterning by Dscam and synaptic partner matching in the *Drosophila* antennal lobe. *Nat Neurosci* **9**: 349–355.
- Zhu S, Lin S, Kao CF, Awasaki T, Chiang AS, Lee T. 2006b. Gradients of the *Drosophila* Chinmo BTB-zinc finger protein govern neuronal temporal identity. *Cell* **127**: 409–422.
- Zou DJ, Chesler AT, Le Pichon CE, Kuznetsov A, Pei X, Hwang EL, Firestein S. 2007. Absence of adenylyl cyclase 3 perturbs peripheral olfactory projections in mice. *J Neurosci* **27**: 6675–6683.
- Zou DJ, Feinstein P, Rivers AL, Mathews GA, Kim A, Greer CA, Mombaerts P, Firestein S. 2004. Postnatal refinement of peripheral olfactory projections. *Science* **304**: 1976–1979.

Cold Spring Harbor Perspectives in Biology

Topographic Mapping—The Olfactory System

Takeshi Imai, Hitoshi Sakano and Leslie B. Vosshall

Cold Spring Harb Perspect Biol 2010; doi: 10.1101/cshperspect.a001776 originally published online June 16, 2010

Subject Collection [Neuronal Guidance](#)

Mechanisms and Molecules of Neuronal Wiring: A Primer

Alex L. Kolodkin and Marc Tessier-Lavigne

Guidance Molecules in Axon Pruning and Cell Death

Pierre Vanderhaeghen and Hwai-Jong Cheng

Initiating and Growing an Axon

F. Polleux and William Snider

Navigating Intermediate Targets: The Nervous System Midline

Barry J. Dickson and Yimin Zou

Cellular Strategies of Axonal Pathfinding

Jonathan Raper and Carol Mason

Guidance Molecules in Axon Regeneration

Roman J. Giger, Edmund R. Hollis II and Mark H. Tuszynski

Signaling from Axon Guidance Receptors

Greg J. Bashaw and Rüdiger Klein

Visual Map Development: Bidirectional Signaling, Bifunctional Guidance Molecules, and Competition

David A. Feldheim and Dennis D. M. O'Leary

Wiring the Brain: The Biology of Neuronal Guidance

Alain Chédotal and Linda J. Richards

Guidance Molecules in Synapse Formation and Plasticity

Kang Shen and Christopher W. Cowan

The Growth Cone Cytoskeleton in Axon Outgrowth and Guidance

Erik W. Dent, Stephanie L. Gupton and Frank B. Gertler

Topographic Mapping—The Olfactory System

Takeshi Imai, Hitoshi Sakano and Leslie B. Vosshall

Self-avoidance and Tiling: Mechanisms of Dendrite and Axon Spacing

Wesley B. Grueber and Alvaro Sagasti

Trafficking Guidance Receptors

Bettina Winckler and Ira Mellman

Axon Guidance Molecules in Vascular Patterning

Ralf H. Adams and Anne Eichmann

Human Genetic Disorders of Axon Guidance

Elizabeth C. Engle

For additional articles in this collection, see <http://cshperspectives.cshlp.org/cgi/collection/>

**All Modifications and
Oligo Types Synthesized**

Long Oligos • Fluorescent • Chimeric • DNA • RNA • Antisense

Oligo Modifications?

Your wish is our command.