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Review

Development of Continuous and Discrete Neural Maps

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Two qualitatively different kinds of neural map have been described: continuous maps exemplified by the visual retinotopic map, and discrete maps exemplified by the olfactory glomerular map. Here, we review developmental mechanisms of retinotopic and olfactory glomerular mapping and discuss underlying commonalities that have emerged from recent studies. These include the use of molecular gradients, axon-axon interactions, and the interplay between labeling molecules and neuronal activity in establishing these maps. Since visual retinotopic and olfactory glomerular maps represent two ends of a continuum that includes many other types of neural map in between, these emerging general principles may be widely applicable to map formation throughout the nervous system.

1. Introduction

The use of space to encode information is a fundamental organizational principle of the nervous system. Neural maps are used in all sensory modalities, motor control, and many places in between (see other articles in this issue). Two qualitatively different kinds of neural maps have been described: continuous and discrete (Figure 1). In continuous neural maps, nearby neurons in the input field connect with nearby neurons in the target field, thereby preserving spatial order (Figure 1, top). An example is the retinotopic map in the visual system, where a two-dimensional image on the retina is recast in higher visual processing areas in the brain. In discrete neural maps, the spatial organization of the one field reflects discrete qualities, rather than the spatial organization, of neurons in the other field (Figure 1, bottom). An example is the glomerular map in the olfactory system, where olfactory receptor neurons that express the same odorant receptors project to the same glomerular units in the brain, creating a spatial map of discrete information in the target.

Most neural maps either resemble one of these two extremes or fall in between. Auditory projections form a continuous tonotopic map, which is comparable to the retinotopic map. Taste systems use discrete channels for different taste modalities, resembling the olfactory map. Somatosensory and motor maps contain both continuous and discrete components; for example, somatosensory maps typically consist of a continuous representation of the body surface, but embedded in this are discrete units such as whisker barrels. Higher visual maps can contain both a continuous representation of visual space and discrete embedded columnar units to represent features such as ocular dominance and direction of motion. Historically, the term "topographic" was used to describe continuous maps exemplified by the retinotopic map; but this term has become extended to other maps with a spatial component. We use here the term "continuous" to make a distinction with discrete maps. When we use "topographic" in this review, we treat it according to its historic meaning (i.e., synonymous with "continuous").

How are neural maps formed during development? Are there common principles used in the formation of all neural maps? Here, we review recent advances on mechanisms of map development. We focus specifically on the retinotopic map in the visual system (best-studied continuous map) and the glomerular map in the olfactory system (best-studied discrete map). By comparing these two systems that represent extremes in the spectrum of continuous and discrete maps, we attempt to identify emerging common principles, as well as diversity of solutions, that may apply to the formation of other neural maps throughout the nervous system.

2. Development of Retinotopic Maps

Visual maps provide the prototypic example of continuous topographic mapping. Light falling on the retina creates a two-dimensional image of the outside world. How this information is then transferred to the brain has long been of interest, and the idea that the image would be transferred in a spatially intact form to visual centers in the brain was first proposed in the 17th century (Jacobson, 1991). This transfer is now known to involve a topographic organization of axons (Figure 2). In discussing mechanisms for map formation, we will first focus on a vertebrate system, the well-characterized projection from the retina to the midbrain tectum (or its mammalian equivalent, the superior colliculus), followed by a discussion of retinotopic development in *Drosophila*.

2.1 Retinotectal Map Formation: Graded Positional Labels

In explaining how a continuous topographic map could develop, Sperry first proposed in his chemoaffinity theory that maps such as the retinotectal projection could be specified by complementary positional labels in gradients (Sperry, 1943, 1963). These labels would mark position across both the retina and tectum, and the projecting

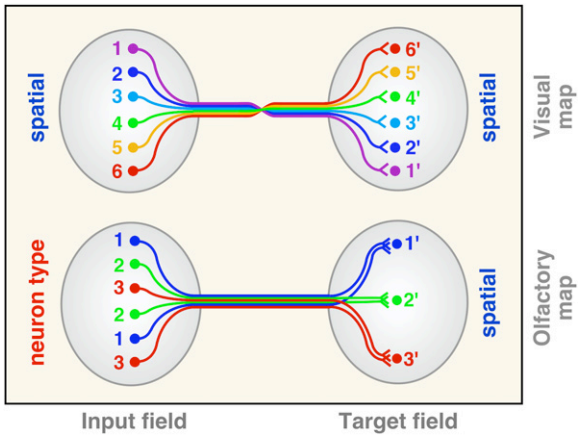

Figure 1. Continuous and Discrete Neural Maps
(Top) Schematic of continuous neural map exemplified by the retinotopic projections of the visual system. Nearby neurons in the input field connect with nearby neurons in the target field, thereby preserving the spatial order of the visual image.
(Bottom) Schematic of discrete neural map exemplified by the glomerular map in the olfactory system. Spatial organization in the target field reflects type rather than position of input neurons; each color represents olfactory receptor neurons that express a common odorant receptor, which converge their axonal projection to a common glomerulus.

retinal axons would find their correct position in the tectal gradient, based on their position in the retinal gradient.

The ephrin-As and their EphA receptors were subsequently identified as molecules that fulfill all the major criteria to be complementary graded mapping labels. First, the receptors and ligands show appropriate expression patterns in the retinotectal system, being in complementary gradients across both the retina and the tectum (Figure 2A) (Cheng et al., 1995; Drescher et al., 1995). Second, when tested in vitro the ephrins act as guidance cues for retinal axons (Drescher et al., 1995; Nakamoto et al., 1996). And third, when tested in vivo by both gain- and loss-of-function approaches, both the ligands and receptors can change the map position where retinal axons project (Nakamoto et al., 1996; Frisén et al., 1998; Brown et al., 2000; Feldheim et al., 2000). The ephrins currently remain as the only molecules validated by all these tests as graded mapping labels. Importantly, the functional assays all show differential responses by axons from different positions in the projecting area, an essential property if a molecule is to act as a topographic label.

For the development of a two-dimensional map, it is necessary to have labels along at least two distinct axes. The ephrin family appears to neatly provide a solution to this problem. The ephrins and Eph receptors are divided into A and B subfamilies, which show preferential binding interactions within a subfamily (Eph Nomenclature Committee, 1997; Klein, 2004). While the A subfamily acts as mapping labels for the retinotectal anteroposterior axis, the B subfamily has a labeling function along the dorsoventral axis (Figure 3A) (Holash and Pasquale, 1995; Braisted et al., 1997; Hindges et al., 2002; Mann et al., 2002).

Although topographic mapping is usually described in terms of labeling along two axes at right angles, there is no inherent necessity for the labels to be simple monophasic gradients along orthogonal axes. Indeed, in the original chemoaffinity theory Sperry predicted that in binocular species the anteroposterior axis would be mapped by labels in a central-to-peripheral gradient. His reasoning was that this would allow the temporal side of one eye and the nasal side of the other eye—which in binocular species view the same part of the visual field—to map to the same position in the target (Figure 2B) (Sperry, 1963). Studies on the human visual system have now shown that retinal EphA receptors form a gradient that is low at nasal and temporal extremes, rising to a high point in the central retina (Lambot et al., 2005). In addition to providing an explanation for binocular mapping, this result emphasizes that it is worth bearing in mind that other topographic maps may not be labeled by simple Cartesian coordinate systems.

2.2 Positive and Negative Effects of Ephrins

While the general concept of chemoaffinity labels can explain the formation of a continuous topographic map, Gierer pointed out in the 1980s that a single type of guidance gradient along each axis is not enough: whether such a gradient were repellent or attractant, the result would simply be accumulation of all the axons at one end of the target (Gierer, 1987). Gierer therefore proposed that axons must detect two opposing forces in the target. These forces might take various forms, for example an attractant and a repellent, opposing gradients of repellents, or a single molecule with both attractant and repellent properties. Each axon would then come to rest at the point where the opposing forces cancel out, allowing formation of a continuous topographic map where different axons would terminate at different points.

Ephrin-As are distributed in the tectum in a posterior > anterior gradient, and initial studies led to a model where they would act as repellents (Flanagan and Vanderhaeghen, 1998; O'Leary et al., 1999). This model leads to a prediction: if the counterbalancing force in the target were a fully independent gradient, then removal of a repellent ephrin-A gradient should cause a tendency for axons to shift toward abnormally posterior positions, or in the case of extreme nasal axons would cause no shift. When ephrin-A gene knockouts were examined, temporal retinal axons were indeed found to form ectopic arbors in abnormally posterior locations, as anticipated (Frisén et al., 1998; Feldheim et al., 2000). However, examination of nasal axons led to a surprise, since they undergo a comparable degree of shift, but in the opposite, anterior direction (Figure 3C) (Feldheim et al., 2000). These results argue against a simple model where a fully independent gradient provides the counterbalancing force.

Although the first tests had detected only repellent effects of ephrins (Drescher et al., 1995; Nakamoto et al., 1996)—and it is still sometimes assumed that ephrins must act as repellents—many subsequent studies have shown that, like a number of other guidance molecules,

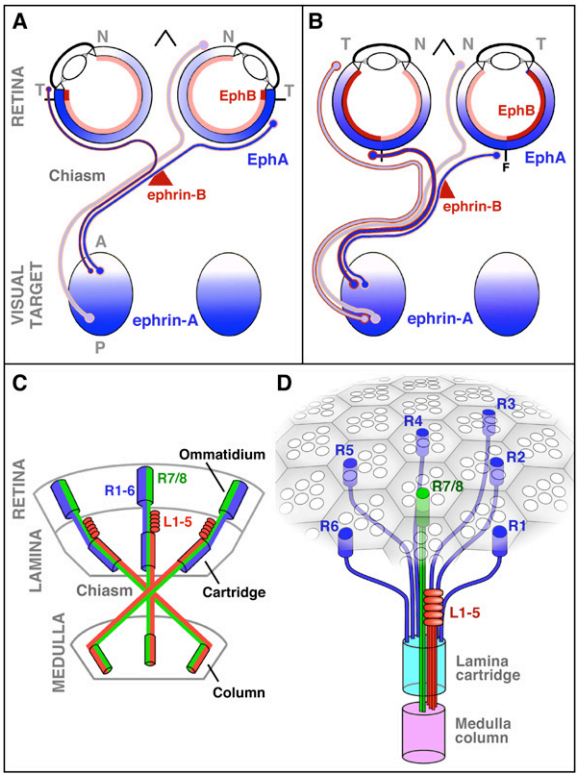

Figure 2. Topographic Organization of Retinal Projections

(A and B) Retinotopic connections in vertebrates. The two-dimensional image on the retina is transferred to visual centers in the brain as a continuous topographic map, with spatial order preserved along both anteroposterior (A-P; illustrated here) and dorsoventral (D-V; not shown) axes. To avoid confusion in binocular species, the A-P axis of the retina is commonly termed nasotemporal (N-T). (A) In species such as mouse and chick, the two eyes view divergent parts of visual space, with little binocular overlap. Axons from each eye predominantly project through the chiasm to the contralateral lobe of the tectum (or its mammalian equivalent, the superior colliculus), forming a map with nasal axons terminating in posterior tectum and temporal axons in anterior tectum. In map development, EphA and ephrin-A molecules (blue) act as graded complementary labels along the N-T and A-P axes. EphB and ephrin-B molecules (red) serve an early function in selective chiasm crossing (illustrated here), in addition to their later function in D-V mapping (see Figure 3). (B) In binocular species such as humans, both eyes point in the same direction. The nasal half of each retina projects contralaterally, while the temporal half projects ipsilaterally. The two eyes form maps in register, with the temporal extreme of one eye and the nasal extreme of the other eye—which view the same point in visual space—mapping to the same position in the target. During development, the retinal EphA gradient is highest in central retinal (F, presumptive fovea) and declines toward the nasal and temporal extremes, providing a coordinate system for the two eyes to map in register. Diagrams are modified from Lambot et al., 2005.

(C and D) Retinotopic connections in *Drosophila*. The retina consists of ~750 ommatidia in an orderly hexagonal array. Each ommatidium contains eight photoreceptor cells, R1–8, in a pattern with R8 located below R7. The retina projects to two ganglia, the lamina and the medulla, which also consist of orderly arrays of units, respectively called cartridges and columns. Each cartridge receives input from the six R1–6 neurons in six adjacent ommatidia, which detect light from a single point in visual space. Projections to the medulla form a chiasm that inverts AP and DV order. Each column in the medulla receives direct input from one R7 and one R8 neuron, as well as input from a cluster of five interneurons located in the lamina, L1–5. The result of this highly stereotyped pattern of local connections is that cartridges and columns each receive input from a single point in visual space and form highly ordered topographically mapped arrays.

ephrins are bifunctional. In assays of cell adhesion, cell migration, and axon guidance, both negative/repellent and also positive/attractant effects have been demonstrated for both ephrin-As and ephrin-Bs (Huynh-Do et al., 1999; Holmberg et al., 2000; McLaughlin et al., 2003a; Weinl et al., 2003; Hansen et al., 2004; Matsuoka et al., 2005; Weinl et al., 2005; Halloran and Wolman, 2006; Zimmer et al., 2007).

A crucial feature of this bifunctionality, in the case of the ephrins, is that it is concentration dependent. When retinal axons respond in vitro to ephrin-A2, low concentrations of the ligand cause a several-fold promotion of outgrowth, whereas high concentrations cause a complete inhibition. Moreover, the balance of positive and negative effects shows a systematically graded dependence on both ephrin concentration and retinal position. These results lead to a model, entirely consistent with Gierer's proposals, where ephrin-As would contribute both positive and negative forces in mapping (Figure 3B) (Hansen et al., 2004).

Ephrin-Bs are also thought to have bifunctional actions on retinal axons, since gene disruption of EphBs produced a phenotype consistent with attraction (Hindges et al., 2002), whereas overexpression of patches of ephrin-B1 in chick caused repulsion (McLaughlin et al., 2003a). Although the nature of the evidence for ephrin-B bifunctionality was quite different from that for ephrin-As, it led to a similar model, where axon branches located too high or too low in the ephrin-B gradient would be respectively repelled or attracted toward their topographically correct D-V position (Figure 3B) (McLaughlin and O'Leary, 2005).

The molecular mechanism for bifunctionality could in principle involve transduction into the axon of attractant signals by some Eph receptors and repellent signals by others. Alternatively, it may involve adhesion at low ligand concentrations, and repellent signal transduction at high ligand concentrations, and current evidence appears consistent with this second model (Holmberg et al., 2000; Hansen et al., 2004; Matsuoka et al., 2005; Halloran and Wolman, 2006). Signaling can also cause proteolytic disruption or endocytosis of the ligand-receptor complex, and this may help to explain how the Eph-ephrin interaction can promote adhesion at low concentrations (or low oligomerization states) and cause repellent signaling at high concentrations (Hattori et al., 2000; Zimmer et al., 2003; Janes et al., 2005).

Along both the A-P and D-V axes, bifunctionality is organized such that axons can be attracted by a low concentration of ephrin and repelled by a high concentration (Figure 3B). However, a difference is that the high point on the Eph gradient maps to the low point on the ephrin-A gradient, but to the high point on the ephrin-B gradient (Figure 3A). We suggest that this does not necessarily reflect a fundamental difference in the underlying molecular

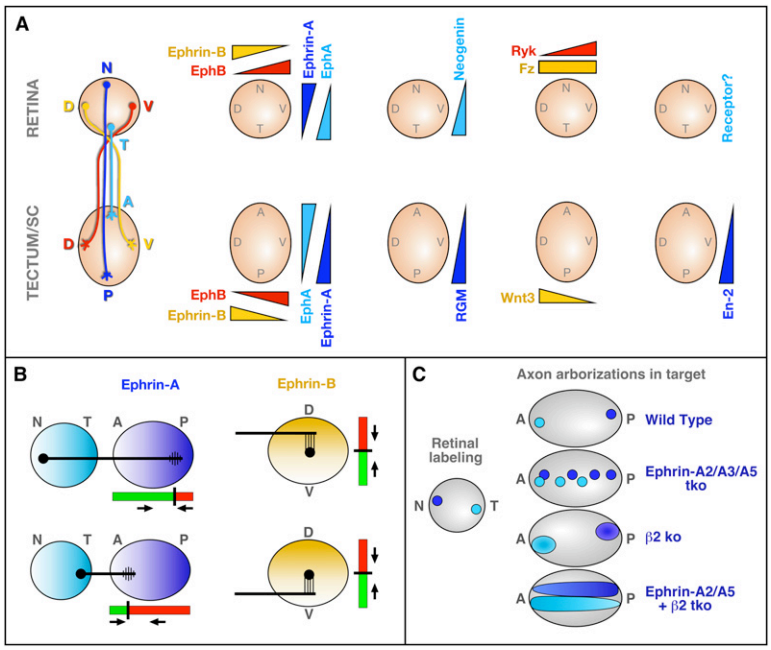

Figure 3. Gradients, Bifunctional Guidance Mechanisms, and Neural Activity in the Retinotectal Projection

(A) Gradients of guidance cues and their receptors in the retinotectal system. Map topography is shown at the left. Axons map from the retina to the tectum of the midbrain, also known as the superior colliculus in mammals. A, anterior; P, posterior; N, nasal; T, temporal; D, dorsal; V, ventral (D and V in the tectum are sometimes termed medial and lateral). All of the illustrated tectal molecules display topographic specificity (producing distinct responses by axons from different parts of the retina), although only the ephrins/Ephs are known to be required as tectal guidance labels in vivo. Ephrin and Eph gradients are based on studies of chick, mouse, and *Xenopus*. Each Eph/ephrin gradient illustrated is typically a composite of more than one molecule (for details, see McLaughlin and O'Leary, 2005); for example, the tectal ephrin-A gradient contains ephrin-A2, -A3, and -A5. RGM and its receptor neogenin are in complementary gradients. Wnt3 is graded in the tectum, and its receptors are in the retina: Ryk is a tyrosine kinase receptor that mediates repulsion in vitro, while Frizzled (Fz) proteins are seven-transmembrane receptors and mediate the attraction seen at lower Wnt3 concentrations. En-2 attracts nasal and repels temporal axons in vitro, apparently by crossing the cell membrane, implying that it has one or more graded intracellular retinal receptors.

(B) Bifunctional guidance mechanisms. Ephrin-As, ephrin-Bs, and Wnt3 all show bifunctional effects on retinal axons. Low concentrations cause positive/attractant effects whereas high concentrations cause negative/repellent effects. This leads to a model for mapping along both A-P and D-V axes (left and right diagrams) where axons find their final position at the neutral point between attraction (green bars) and repulsion (red bars). On the A-P axis, ephrin-As are proposed to specify a neutral point that varies depending on the retinal position of axon origin (upper and lower diagrams) (Hansen et al., 2004). On the D-V axis, the termination zone (black circle) is formed in birds and mammals by topographic guidance of collateral axon branches. For an axon located dorsal to its final topographically correct position, the collateral branches are proposed to be repelled down the ephrin-B gradient (upper diagram), whereas for an axon located ventral to the correct position, the collateral branches would be attracted up the gradient (lower diagram) (McLaughlin and O'Leary, 2005). A difference is that for ephrin-As the high point on the retinal receptor gradient maps to the low point on the tectal ligand gradient, whereas for ephrin-Bs the high point on the receptor gradient maps to the high point on the ligand gradient (Figure 3A). However, this need not reflect a fundamental difference in the underlying molecular explanation for concentration-dependent bifunctionality (see text).

(C) Respective roles of graded labels and neural activity. In a wild-type mouse, focal dye labeling of nasal or temporal retinal axons shows a tight focus of axon arborization at the topographically corresponding position in the target. In ephrin-A double and triple gene knockout mice, both nasal and temporal axons form ectopic terminations scattered along the A-P axis, but they still arborize in tight foci. Mice mutant for the β-2 subunit of the nicotinic acetylcholine receptor, which lack early spontaneous retinal waves, show arborizations that are abnormally diffuse, but located in the topographically correct area. When the ephrin-A and β2 mutations are combined, axons form a single diffuse arborization that can fill most of the A-P axis. Some topographic bias remains, suggesting the presence of additional graded labels. These results support a model where topographic order is set up by graded labels and refined by patterned neural activity.

explanation of bifunctionality. Instead, it may only reflect the quantitative response curves of each receptor to adhesion and repulsion. If increasing receptor concentration causes the repulsion term to rise above the adhesion term, then high receptor would map to low ligand, but if it causes the adhesion term to rise above the repulsion term, then high receptor would map to high ligand.

Interestingly, a topographically specific guidance molecule in a different family, Wnt3 (Figure 3A), which will be discussed further below, also shows this property of concentration-dependent bifunctionality. Furthermore, like ephrin-A2, the balance of positive versus negative effects of Wnt3 in vitro was found to vary systematically with both ligand concentration and retinal position (Schmitt et al., 2005). For both ephrin-B1 (McLaughlin and O'Leary, 2005) and Wnt3 (Schmitt et al., 2005), the gradients appear to act in the same direction, with higher concentrations located dorsally and proposed to cause repulsion, in which case it does not seem that they act as gradient forces opposed to one another in any simple way; instead, they may act as a partially redundant system of cooperating labels to make dorsoventral mapping more robust.

Concentration-dependent bifunctionality, which is seen in ephrin-As, ephrin-Bs, and Wnt3, can provide a unified mapping mechanism along both A-P and D-V axes and appears to be a common feature of graded mapping cues. This can allow them to fulfill Gierer's postulate by not merely pushing or pulling axons in one direction, but rather by specifying a position where each axon comes to rest in the gradient.

2.3 Eph and Ephrin Countergradients

A notable feature of ephrin and Eph expression patterns is that they generally take the form of countergradients within an area. In the retina, for example, EphA receptors are found in a temporal > nasal gradient, while ephrin-A ligands are found in a nasal > temporal gradient

(Figure 3A). There are several potential roles for this countergradient expression.

First, it has been proposed that ephrin-A expressed in retinal neurons would interact in *cis* with EphA receptors, downregulating their action in nasal retina. This idea is supported by compelling evidence from several studies. Ephrin-A overexpression in the retina causes temporal axons to lose their sensitivity to ephrin-A repulsion (Hornberger et al., 1999). Correspondingly, ephrin-A gene knockout greatly increases the sensitivity of explanted nasal retinal axons to ephrin-A repulsion (Feldheim et al., 2000). Biochemical studies have shown that ephrin-As cause downregulation of EphAs when expressed in the same cell, and this *cis* interaction is mediated by a domain in the receptor that is distinct from the binding site for *trans* receptor-ligand interaction (Yin et al., 2004; Carvalho et al., 2006). By downregulating EphA receptors preferentially at the nasal extreme of the retina, the effect of the *cis* interaction may be to make the functional gradient of retinal EphA receptors steeper, perhaps serving to enhance the precision of mapping.

A second role for the countergradients comes from the ability of ephrins and Eph receptors to signal bidirectionally, with a ''forward'' signal transduced into the cell carrying the Eph and a ''reverse'' signal transduced into the cell carrying the ephrin (Klein, 2004). As a result, ephrins on projecting axons and Eph receptors in the target can also contribute to mapping. Along the retinotectal anteroposterior axis, EphA7 is in an anterior > posterior gradient in the target and can repel mouse retinal axons in vitro and suppress arborization in vivo (Rashid et al., 2005). Along the dorsoventral axis, EphB receptors are expressed in a ventral > dorsal gradient in the *Xenopus* tectum and can have an attractant effect in vivo and in vitro for dorsal retinal axons (Mann et al., 2002).

Taken together, the previous two paragraphs raise a question: if ephrins interact in *cis* with Ephs and downregulate them, can both Ephs and ephrins be active as receptors in the same axons? One potential resolution to this paradox comes from a study of motor neurons, where immunolocalization experiments provided evidence that ephrin-As and EphAs do not interact in *cis*, but rather segregate into separate microdomains where they may signal independently (Marquardt et al., 2005). Superficially, these results seem to contradict the studies described above finding that interactions do occur in *cis*, including at the cell surface. While the explanation for these differences is not yet clear, it seems possible that both models are correct. For example, different neuronal types may show *cis* or *trans* interactions. Alternatively, a subset of the molecules on a single axon may interact in *cis* and mediate downregulation, while another subset may segregate and act independently as guidance receptors.

Finally, a third reason for the expression of both Ephs and ephrins within the same area is that most regions of the nervous system act both as projecting areas and as target areas. The expression of countergradients of both ephrins and Eph receptors may be important in allowing a single area to serve as both the recipient of mapped incoming axons and the origin of mapped outgoing axons. This feature may be critical for the serial (Feldheim et al., 1998; Cang et al., 2005), parallel (Feldheim et al., 1998), and reciprocal (Torii and Levitt, 2005) transfer of topographically mapped information among multiple interconnected areas of the nervous system (Flanagan, 2006).

2.4 Axon-Axon Competition: Filling the Target

A notable feature of continuous topographic mapping is that projecting axons tend to smoothly fill up the target. This is seen not only in normal development, but even after fairly drastic experimental manipulations such as removal of half the retina or tectum, which can be followed by gradual map expansion or compression (Fraser and Hunt, 1980; Goodhill and Richards, 1999), or after genetic overexpression or deletion of mapping labels (Brown et al., 2000; Feldheim et al., 2000). These results show that mapping labels do not act by dictating an invariant matching up of concentrations along the gradients, but rather by determining the relative position of axons. The tendency to fill the available space is then explained by axon-axon competition. This competition could in principle result from direct axon-axon interactions or could result from axons competing for one or more limiting factors in the target.

While the mechanism for competition is not well understood at the molecular level, there are some candidates. The neurotrophin BDNF and its receptor trkB are expressed in the tectum and retina, respectively, although not in gradients. Increasing or decreasing the amount of BDNF in the tectum causes expansion or reduction, respectively, in the size of retinal axon terminal arbors (Cohen-Cory and Fraser, 1995). These properties seem to fit the description of a limiting factor in the target that axons may compete for, although a role for BDNF in filling the target has not been shown directly. Another candidate is L1, a member of the immunoglobulin-related cell adhesion molecule (IgCAM) family. L1 gene disruption causes abnormalities in mapping of both temporal and nasal axons when traced by focal dye injections. This mutant also has the unusual property that after dye filling of the entire retina, axons do not fill the tectum smoothly, but instead leave large irregularly shaped patches with a reduced density of axon terminals (Demyanenko and Maness, 2003). L1 is thus required to smoothly fill the target and is an excellent candidate to mediate axon-axon competition.

It seems very possible that axon competition may be mediated by several different molecules in combination, potentially including IgCAMs, neurotrophins, and other factors that may be limiting in the target or mediate repulsion (see sections 2.7 and 3.3 for molecules that may have analogous functions). These molecules could act as genetically specified labels or via activity-dependent mechanisms (see section 2.6); for instance, transcription of BDNF is regulated by neural activity (West et al., 2001). Indeed, a role for neural activity in competition is supported by the finding that growth of retinotectal axon arbors is inhibited when their activity is suppressed below that of active neighbors (Hua et al., 2005). Whatever the specific molecules and mechanisms involved, it is not difficult to

see the advantages of a mapping mechanism that operates by a combination of graded positional labels that determine relative position, together with axon competition. This combination can provide a flexible and robust system to generate a smooth and orderly topographic map throughout the target, despite changes in parameters such as the concentration of the labeling molecules or the size and shape of the map, when they vary during development or evolution.

2.5 Additional Graded Molecules in the Retinotectal Projection

Removal of all the known tectal ephrin-As by double and triple gene knockout, in combination with activity disruption almost completely abolishes topographic order, confirming the importance of ephrins in mapping. However, even in these conditions, some topographic bias remains, supporting the presence of additional labeling mechanisms (Feldheim et al., 2000; Pfeiffenberger et al., 2006). One candidate is RGM, which is in a posterior > anterior tectal gradient and has topographically specific repellent effects on temporal axons in vitro (Monnier et al., 2002). No retinotectal mapping phenotype was detected in RGM knockout mice (Niederkofler et al., 2004), although it remains possible that a role could have been masked by redundancy with other cues, such as the ephrin-As. Sema3D is expressed in the ventral zebrafish tectum, and although it is not clear whether this distribution is graded or discrete, changing the levels of Sema3D causes abnormal mapping of ventral retinal axons (Liu et al., 2004).

In addition to these cues that were initially identified as guidance molecules, other studies have shown effects on retinal axon guidance by two families of molecules that had previously been best known for functions quite different from guidance. The Wnts are cell-cell signaling molecules that have been studied extensively for their effects on cell fate, including actions as graded morphogens. In the retinotectal system, Wnt3 is expressed in a dorsal > ventral gradient in the target (Figure 3A). It was found to have topographically specific effects on retinal axons in vitro, with axon inhibition mediated by the Ryk receptor and promotion mediated by Frizzled receptors, and it was also found to affect mapping in vivo (Schmitt et al., 2005). While their requirement has not yet been tested by gene disruption, these studies support a role of Wnt3 and its receptors in dorsoventral mapping.

An even more striking example of a molecule known for very different functions having axon guidance activity came from a study of the homeodomain transcription factor En-2. A number of previous studies had shown that En proteins are expressed in the tectum in a posterior > anterior gradient and regulate tectal cell fate, including the downstream expression of ephrin-As (Logan et al., 1996; Retaux and Harris, 1996). It had also been shown that En misexpression causes mapping abnormalities, which were explained in terms of its effect on ephrin-A expression (Friedman and O'Leary, 1996; Logan et al., 1996). Although homeoproteins are best known as nuclear factors, studies by Prochiantz and colleagues over the last 15 years have shown that they can be secreted and efficiently taken up by cells in culture, suggesting the potential to act in cell-cell signaling (Joliot and Prochiantz, 2004). Soluble En-2 was therefore tested for a direct effect on axons and was found to act as a guidance factor for *Xenopus* retinal axons in vitro. Moreover, it showed appropriate topographic specificity, attracting nasal axons and repelling temporal axons (Brunet et al., 2005). In the future, it will be interesting to see whether En proteins are transferred to axons in intact tissues and to test the requirement for En as a guidance cue in vivo, which may be inherently challenging in view of its known function in regulating tectal cell fate. Nevertheless, the studies on Wnt3 and En-2 provide new examples of the emerging principle that positional information gradients provide coordinate systems that can be interpreted to regulate essentially any cell function, depending on the receptor systems used to decode this positional information (Osterfield et al., 2003).

2.6 Activity-Dependent Refinement

In explaining the development of neural connectivity, chemoaffinity labels versus neural activity were historically viewed as competing theories (Jacobson, 1991). However, a consensus has now emerged where the two mechanisms are both important and play complementary roles. In the retinotectal system, graded labels initially set up the topographic order of the map, whereas neural activity refines the map to increase its precision (Zhang and Poo, 2001; Ruthazer and Cline, 2004; McLaughlin and O'Leary, 2005; Torborg and Feller, 2005).

The theory underlying most studies of neural activity in mapping is Hebb's proposal that when a pre- and postsynaptic neuron fire at similar times, the connection between them is reinforced (Hebb, 1949). This could lead to refinement of a continuous topographic map if neurons that are adjacent in the projecting area fire in a correlated manner, efficiently triggering an action potential in the postsynaptic neuron and therefore strengthening their connection to it. On the other hand, a neuron located more distantly would show less correlated activity, and its connection to the same postsynaptic neuron would not be strengthened, or may even be weakened. The correlated activity necessary for this Hebbian mechanism could result from visual images falling on the retina, in species such as fish and amphibians, which are visually active while the map forms. In birds and mammals, the correlation may instead be provided by waves of activity that are found to sweep across the retina spontaneously during map development (Meister et al., 1991; Wong et al., 1993).

One line of evidence for the role of neural activity in the retinotectal system has come from inhibitors of neural activity. The use of activity blockers such as tetrodotoxin showed that activity is not required to establish the basic topographic layout of the map (Harris, 1980) but is required for refinement, narrowing down the area covered by axonal connections in the target (Schmidt and Edwards, 1983; Kobayashi et al., 1990). The use of more specific inhibitors shows the NMDA receptor is involved in this refinement (Cline, 1998).

While these studies show that activity is required, they do not address whether its role is permissive or instructive. If the role is to be instructive, a key factor is to show a requirement for suitable patterning of the neural activity. One approach to this has been to force two eyes in an amphibian to innervate the same tectal lobe. The result is a segregation of their connections into ocular dominance bands, and since the two eyes presumably have the same chemoaffinity labels, it can be inferred that the segregation results from correlated activity within each eye (Constantine-Paton and Law, 1978; Ruthazer et al., 2003). A second approach has been to raise fish in stroboscopic illumination, so that retinal neurons fire in a coordinated manner, but independently of their retinal position, and this is found to cause a loss of map refinement (Schmidt and Eisele, 1985). Another approach has been to place electrodes simultaneously in the *Xenopus* retina and tectum. In this way it could be shown that the synaptic connection between the pre- and postsynaptic cells is strengthened or weakened, respectively, when the presynaptic neuron fires within a narrow time window before or after the postsynaptic neuron, providing direct evidence for the basic Hebbian proposal (Zhang et al., 1998). These varied lines of evidence provide strong support for an instructive role of neural activity.

In assessing the relative contributions of labels and activity, genetic studies have been informative (Figure 3C). Following disruption of ephrin-A genes, retinal axons labeled by focal dye injection make connections that are scattered along the anteroposterior axis of the target, but nevertheless form tight termination zones (Frisén et al., 1998; Feldheim et al., 2000). In contrast, mice with a disruption of the β-2 subunit of the neuronal nicotinic acetylcholine receptor, which do not display spontaneous retinal waves during the early phase of map refinement, form retinal axon connections in the topographically correct place, but abnormally diffuse (McLaughlin et al., 2003b; Chandrasekaran et al., 2007). When the ephrin-A mutations and the β-2 mutation are combined, the result is labeling of a single diffuse termination zone covering most of the target (Pfeiffenberger et al., 2006).

Current studies thus support a model where graded labels and patterned neural activity have distinct instructive roles in respectively setting up topographic order and refining it. However, the two processes have interesting areas of overlap; for example, it was recently shown that retinal activity has a permissive role in ephrin signaling during retinotectal map formation (Nicol et al., 2007), and the ephrins are known to act in synapse formation and may in turn regulate activity (Yamaguchi and Pasquale, 2004). Such observations suggest the potential for interesting mechanistic links at the interface between label- and activity-based mapping.

2.7 Retinotopic Mapping in Drosophila

Although the fly eye has a very different structure from its vertebrate counterpart, it shares the hallmark property of projecting to visual centers in the brain as a continuous topographic map. Each eye in *D. melanogaster* consists of a crystalline hexagonal array of approximately 750 ommatidia, each containing eight photoreceptor neurons, R1–8 (Figures 2C and 2D). Each ommatidium sends its axons to two layers, the lamina and the medulla in the brain, forming topographic maps. The lamina and medulla are themselves highly organized, containing an array of units known respectively as cartridges and columns. The axonal connections between these units form in a highly stereotyped pattern so that each cartridge and column corresponds to a single point in external visual space (Figure 2D) (Clandinin and Zipursky, 2002; Mast et al., 2006; Ting and Lee, 2007). The fly visual system has been a rich source for the identification of axon guidance mechanisms; here, we focus on mechanisms to specify topographic order.

The formation of a topographic map where neighboring ommatidia project to neighboring targets in the lamina is thought largely to reflect the spatiotemporal order of ommatidial assembly. As the morphogenetic furrow sweeps across the eye from posterior to anterior, ommatidia differentiate and send out successive rows of axons. The axons themselves organize the lamina, delivering two signaling molecules, Hedgehog and Spitz. These signals induce proliferation and differentiation of lamina neurons, which then associate with the incoming axons to form cartridges (Huang and Kunes, 1996; Huang et al., 1998). This mechanism fulfills at least two purposes. First, it can ensure a matching number of units in the retina and its target. And second, by maintaining the spatiotemporal order of successive rows of axons, the result is generation, at least along the anteroposterior axis, of a topographically mapped organization.

In explaining the local targeting of individual axons to their correct target units, evidence for an important spatial component comes from embryological manipulations. For example, rotating a single ommatidium causes a corresponding rotation in the projection pattern of its axons, indicating that the specificity of R cell axon trajectories is largely determined by the direction of axon outgrowth rather than by specific labels in the target (Clandinin and Zipursky, 2000).

A number of genes have been identified that are required for the axons to project in an organized manner (Clandinin and Zipursky, 2002; Mast et al., 2006; Ting and Lee, 2007). Of particular interest for their role in mediating axon-axon interactions are cell-surface molecules in the cadherin and IgCAM families. Flamingo, a nonclassical cadherin, is required for the correct local topography of R axon projections in the lamina and medulla and probably acts at least in part by homotypic repulsive interactions to ensure correct spacing of the axons (Lee et al., 2003; Senti et al., 2003). In a recent study, Dscam2, a member of the IgCAM family related to the highly diverse recognition molecule Dscam, was suggested to be a homophilic repulsion molecule, and when the Dscam2 gene was specifically deleted from either the L1 axon or its neighbors, the L1 axon no longer targeted to a single column in the medulla but instead could innervate several neighboring columns, disrupting the normal regularly tiled pattern of connections (Millard et al., 2007). These studies show

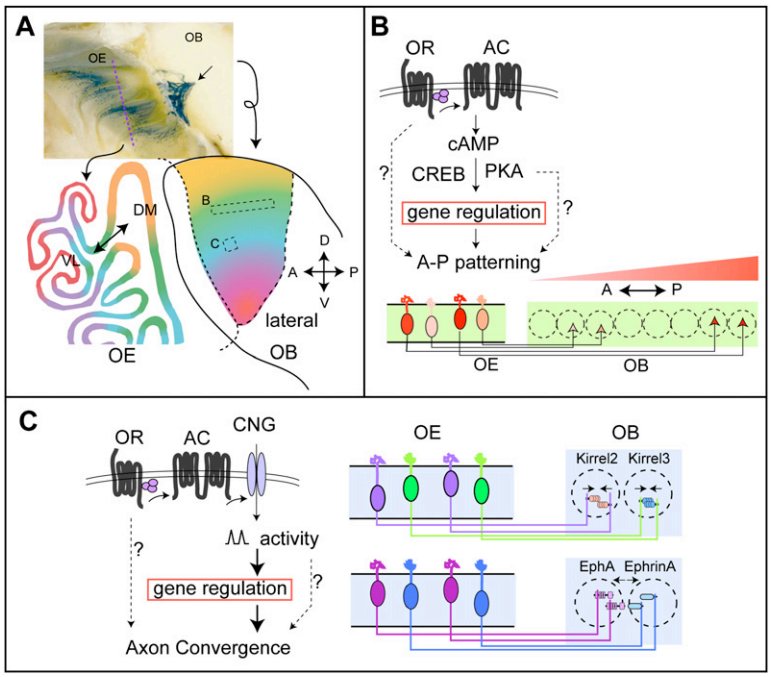

Figure 4. Organization and Development of the Mouse Olfactory Map

(A) (Top) Thousands of olfactory receptor neurons (ORNs) in the olfactory epithelium (OE) that express a common odorant receptor (OR) converge their axonal projections onto the same glomerulus (arrow) in the olfactory bulb (OB). Adapted from Mombaerts et al. (1996). (Bottom) ORNs that express a given OR are distributed within a band along the dorsomedial (DM) to ventrolateral (VL) axis in the OE and project their axons to corresponding color-matched positions along the dorsal-ventral (D-V) axis of the OB. According to data from Miyamichi et al. (2005). Dotted rectangles correspond to OB schematics in (B) and (C) as indicated. A, anterior; P, posterior; D, dorsal; V, ventral. The A-P axis in bottom left (nasal epithelium) is orthogonal to the plane shown.

(B) Basal level G protein (three purple subunits) coupling of individual ORs, through the adenylate cyclase activation of cAMP, PKA, and CREB, induces gene expression that contributes to ORN axon targeting globally along the anterior-posterior (A-P) axis. Specifically, putative axon guidance receptors have been found to be targets of this signaling pathway and exhibit graded distribution along the A-P axis in the OB. According to data from Imai et al. (2006). "?"s denote that OR or cAMP/PKA could regulate global A-P targeting through mechanisms independent of gene regulation.

(C) OR-correlated and activity-dependent expression of homophilic cell adhesion molecules Kirrel2 and Kirrel3, and repulsive axon guidance ligand/receptor pair ephrin-A and EphA, could contribute to local axonal convergence and sorting. According to data from Serizawa et al. (2006). "?"s denote that OR and activity could play additional roles.

a critical role for axon-axon interactions in maintaining precise local topography.

Are global graded labels used in the fly visual system? Although *Drosophila* has a single Eph receptor, no clear evidence has been found for a guidance role in retinotopic mapping (Boyle et al., 2006). A member of the Wnt family, Dwnt4, was found to be expressed in the ventral half of the lamina and was required for ventral targeting of retinal axons in an attractant mechanism dependent on the receptor Dfrizzled2. While this indicates a labeling mechanism analogous to the retinotectal system, the distribution of Dwnt4 appeared to have a sharp boundary, rather than being graded (Sato et al., 2006). It thus remains an open question whether fly retinotopy may use graded chemoaffinity labels. Instead of a primary role for global labels, fly retinotopy may instead rely primarily on the repeated use of local organizing cues, a strategy that may be well suited to its regularly repeated matching arrays.

Finally, the role of neural activity seems to be a difference between fly and vertebrate retinal mapping. In the fly, extensive testing of mutants has so far failed to find a role of neural activity in setting up the pattern of retinotopic connectivity (Hiesinger et al., 2006). Thus, compared with vertebrate systems that exploit neural activity for refinement and adaptation, the highly organized and stereotyped retinal connections in the fly may be specified primarily by precise genetic hardwiring.

3. Development of the Olfactory Glomerular Maps

The olfactory system detects odorants in the chemical world to convey information regarding important matters such as food, predators, and potential mates. How does the brain represent this chemical world and process information that is of special behavioral value? Compared to centuries of studies on the visual map, key insights about the olfactory system organization were obtained only after the molecular cloning of odorant receptors less than two decades ago (Buck and Axel, 1991). Here, we focus on recent advances on the study of olfactory glomerular maps in mice and flies.

3.1 Organization of the Olfactory Systems in Mice and Flies

Following the cloning of odorant receptors, it was found that each olfactory receptor neuron (ORN, also called olfactory sensory neurons) expresses a single odorant receptor (OR). ORNs that express the same odorant receptor (OR)—defined hereafter as belonging to the same ORN class—converge their axonal projections onto the same glomeruli. Remarkably, this organizational principle applies from mammals to insects (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Gao et al., 2000; Vosshall et al., 2000) (Figures 4A and 5A). Thus, the targets of ORN axons are organized as discrete units and form a spatial map. These discrete units represent discrete input channels at the periphery—different ORN classes detecting different odorants in the chemical world. Furthermore, in most species examined to date there is a clustering of glomeruli that respond to odorants bearing particular chemical groups (Mori et al., 2006).

Cell bodies of a given ORN class are distributed widely along the sensory epithelia, intermingled with cell bodies of other ORN classes. One can envision that such

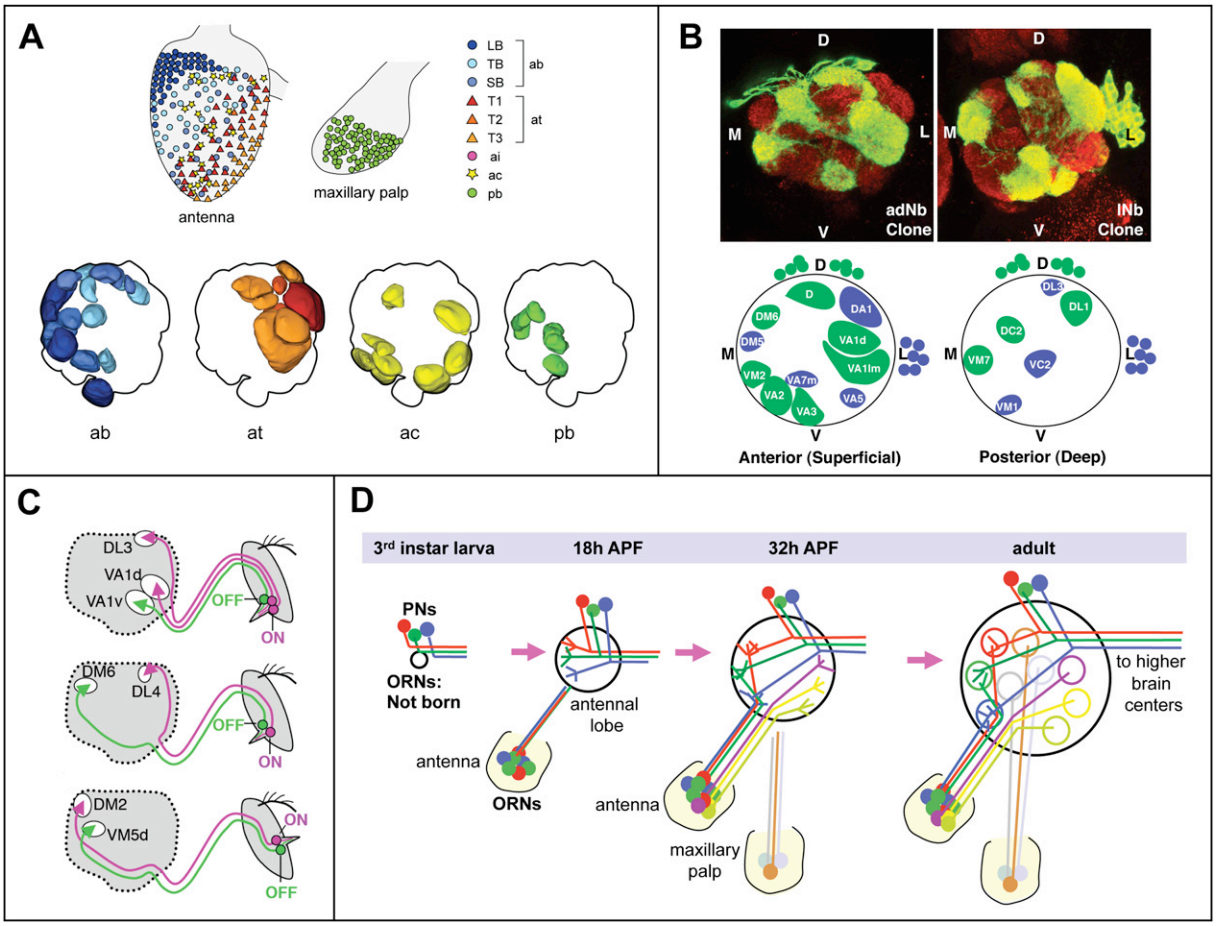

Figure 5. Organization and Development of the Fly Olfactory Map

(A) Corresponding positions of ORN cell bodies in the antenna and maxillary palp (top) and their glomerular targets in the antennal lobe (bottom). Each colored dot represents a sensillum, which houses one to four distinct ORN classes and belongs to a specific type shown on the right. The coarse organization of glomerular position according to sensillum type is evident. Taken from Couto et al. (2005).

(B) (Top) Olfactory projection neurons (PNs) derived from the anterodorsal neuroblast (adNb) project their dendrites to glomeruli that are complementary to those from the lateral neuroblast (lNb). (Bottom) Schematic summary of glomeruli that are targets of PNs derived from adNb lineage (green) and lNb lineage (blue). PN cell bodies are at the periphery of the antennal lobe (large circles), color-coded according to their lineages. Two separate depths along the A-P axis are shown. Modified after Jefferis et al. (2001).

(C) Three examples showing that ORNs belonging to the same sensillum can project to glomeruli that are spatially close or far apart. Each ORN class can be classified as Notch-On or Notch-Off. In the absence of Notch signaling, Notch-On ORNs project axons to glomeruli that are targets of the Notch-Off ORN class within the same sensillum. Taken from Endo et al. (2007).

(D) Summary of key steps in olfactory circuit assembly in *Drosophila*. (1) At the end of the larval stage, adult ORNs are not born yet; all PNs are born and have extended their main axon trunks to higher brain centers but have just started to extend rudimentary dendrites into the area that will become the adult antennal lobe. (2) Between 0 and 18 hr after puparium formation (APF), PN dendrites elaborate and expand within the antennal lobe while ORNs are being born. By 18 hr APF, PN dendrites already occupy the approximate areas of the antennal lobe according to their future glomerular classes (symbolized by different colors); pioneering antennal ORNs have just reached the edge of the antennal lobe. (3) At 32 hr APF, pioneering axons from the maxillary palp (MP) ORNs reach the antennal lobe, which has been patterned by antennal ORN axons in addition to PN dendrites. (4) Synaptic matching gives rise to the adult pattern of connectivity. See text for mechanisms that regulate each of these steps.

distribution could enhance odor detection sensitivity, accuracy, and resistance to local damage. It is often described in the literature that the distribution of cell bodies of ORNs expressing a given OR is random. However, this is not accurate for mice or flies. ORNs in the mouse are distributed in a convoluted two-dimensional nasal epithelium that can nevertheless be simplified with two axes, anterior-posterior (A-P) and dorsomedial-ventrolateral (DM-VL) (Figure 4A). Interestingly, while the distribution of cell bodies belonging to the same ORN class appears "random" along the A-P axis, the distribution along the DM-VL axis is more orderly. Early in situ hybridization experiments identified several discrete zones along the DM-VL axis, with cell bodies of each ORN class distributed in one of these discrete zones (Ressler et al., 1993; Vassar et al., 1993). Recent experiments examining many more ORN classes indicate that OR genes are expressed in multiple overlapping bands along the DM-VL axis, which

on average span one-quarter of the nasal epithelium. Further, there is a strong correlation between the location of the ORN cell bodies along the DM-VL axis of the epithelium and their target glomeruli along the D-V axis of the olfactory bulb, indicating a coarse continuously mapped topography (Miyamichi et al., 2005) (Figure 4A). This begins to smell like the visual map. However, given that there are 1000 ORN classes, each spanning one-quarter of the epithelium, there must be a lot of "salt and pepper" mixing of cell bodies of different ORN classes even along the DM-VL axis.

In *Drosophila*, ORN cell bodies are organized into specialized structures called sensilla on each antenna and maxillary palp. Each sensillum houses one to four ORNs belonging to one to four specific ORN classes in a stereotyped fashion (de Bruyne et al., 1999, 2001). The cell body positions and the glomerular targets for most of the ~50 ORN classes have been mapped (Couto et al., 2005; Fishilevich and Vosshall, 2005). There is coarse organization with regard to the nature of the odor and corresponding sensillar types. For instance, ORNs belonging to the trichoid sensilla, which usually respond to pheromones, cluster their cell bodies in one area of the antenna; basiconic ORNs, which usually respond to fruity odorants, occupy other areas of the antenna. In the antennal lobe, trichoid ORN targets are located in the lateral anterior region of the antennal lobe, whereas basiconic ORNs from the antenna and the maxillary palp target distinct glomeruli in other parts of the antennal lobe (Figure 5A). Beyond this coarse organization, however, cell bodies of individual ORN classes intermingle with those of other ORN classes belonging to the same sensillar type. There is no clear topography apparent at this fine level: for example, two ORN classes that pair their cell bodies within the same sensillum target their axons to distinct glomeruli that can be far apart in the antennal lobe (Figure 5C).

Information from ORNs is carried to higher olfactory centers by second-order olfactory neurons: mitral cells in vertebrates and projection neurons (PNs) in insects. In both mice and flies, each mitral cell or PN sends dendrites to a single glomerulus. However, the precise cellular architecture of these second-order neurons is quite different between the species. Specifically, mitral cells form a layer underneath the glomerular layer in the vertebrate olfactory bulb and tend to innervate a glomerulus close to their cell bodies. Insect PN cell bodies are located adjacent to but outside of the antennal lobe neuropil, which is composed only of dendritic, axonal, and glial processes. In *Drosophila*, there is no discernable correspondence between the positions of PN cell bodies and their glomerular targets. Further, PN dendrite targeting appears to be specified by their lineage and birth order, independent of their synaptic partner ORN axons (Jefferis et al., 2001) (Figure 5B; see below). Indeed, terminal arborization of PN axons in one of the higher brain centers exhibit striking stereotypy corresponding to their glomerular classes (Marin et al., 2002; Wong et al., 2002; Jefferis et al., 2007). Specification of PNs independent of their input neurons could ensure specific olfactory input channels to be stereotypically connected with specific downstream circuits that process qualitatively distinct information, such as food and mating pheromone (Jefferis et al., 2007).

In summary, in both flies and mice, the correspondence of cell body and glomerular positions of ORNs can be described as a mixture of two organizations. First, there is a coarse topography of ORN classes according to cell body positions along the DM-VL axis in the mouse and for different sensillar groups in the fly. Second, there is a position-independent component that correlates with the identity of the ORNs; this applies to the course organization of the A-P axis and the fine-grained glomerular organization of both A-P and D-V axes in mice, and to ORN classes belonging to the same sensillar group and PNs in flies. It is this second organization that is qualitatively different from that of the continuous maps of the visual system. At least in flies there is also the additional challenge that each PN needs to target its dendrites to specific areas of the antennal lobe that will eventually develop into specific glomeruli. Below, we review recent studies that provide insights into the mechanisms of how such wiring specificity is established.

3.2 Mechanisms of ORN Axon Targeting in Mice

In mice, each ORN expresses a single odorant receptor (OR) from ~1000 OR genes. ORNs expressing the same OR converge their axonal projections to a pair of mirror-image glomeruli at the medial and lateral side of the olfactory bulb (Figure 4A). Although there are local variations of glomerular positions (of a few glomerular distance) corresponding to a given ORN class from animal to animal or even from two sides of the same animal (Strotmann et al., 2000), overall targeting is remarkably precise: axons from the same ORN class find each other and converge onto a glomerulus in a rather stereotyped area of the olfactory bulb.

The most intensely studied molecules for ORN axon targeting are the ORs themselves. By replacing the coding sequence of one OR (recipient) with another (donor) using gene targeting, glomerular positions of the resulting ORN axons are altered, often to a position in between the glomeruli corresponding to those of the donor and the recipient (Mombaerts et al., 1996; Wang et al., 1998). These experiments indicate that ORs play an instructive role in ORN targeting. OR proteins have been detected at the terminals of ORN axons (Barnea et al., 2004; Strotmann et al., 2004), suggesting that they might act as guidance receptors or have some other function in the growth cone during axon targeting, but the mechanisms by which they instruct targeting remain enigmatic.

Significant advances have been made recently by genetic engineering of G protein and cAMP signaling pathways in ORNs. Constitutive activation of Gs is sufficient to rescue ORN axon convergence in the absence of a functional receptor (Imai et al., 2006; Chesler et al., 2007). Further, the glomerular positions of a specific ORN class along the A-P axis in the bulb are correlated with different strengths of cAMP/PKA signaling engineered into these ORNs. This difference in cAMP/PKA

signaling further results in differential gene expression likely regulated by the CREB transcription factor (Imai et al., 2006). Putative target genes include neuropilin 1, a receptor for the axon guidance molecule Sema3A previously implicated in restricting ORN axon targeting (Schwarting et al., 2000). Neuropilin 1 proteins, presumably derived from ORN axon terminals, form an A-P gradient in the olfactory bulb correlating with the strength of the cAMP signaling in ORNs whose axons target to different A-P positions (Imai et al., 2006). It has thus been postulated that ORNs expressing a given OR have a defined level of G protein signaling, which can be translated into certain amount of guidance receptor expression, thereby instructing their axons to target defined areas in the olfactory bulb (Figure 4B). This appealing model can account for several peculiar previous findings, including that alterations of OR expression level cause projection shift and that expression of an unrelated G protein-coupled receptor can rescue ORN axon convergence in the absence of a functional OR (Feinstein et al., 2004; Feinstein and Mombaerts, 2004).

The involvement of cAMP signaling in ORN axon targeting was supported by recent adenylate cyclase (Ac3) knockout studies (Col et al., 2007; Zou et al., 2007). In Ac3 mutant mice, gross targeting of ORNs and convergence of specific classes are defective. Consistent with the findings in Imai et al. (2006), targeting to posterior bulb was more severely affected, and neuropilin 1 expression is drastically reduced in Ac3 mutant mice (Col et al., 2007). It remains to be tested whether disruptions of neuropilin 1 and/or other cAMP/CREB targeting genes cause predictable shifts of glomerular position along the A-P axis. In addition to regulating gene expression through CREB, cAMP/PKA signaling is well known for regulating axon guidance responses at the growth cone (Ming et al., 1997). Since ORs are present at the ORN axons and terminals (see above), cAMP/PKA signaling can in principle also differentially modulate growth cone responses to the same environmental cues in different ORN classes.

Given that many values of A-P positioning need to be specified for precise targeting, it remains to be determined whether a gradation of G protein/cAMP signaling levels alone could account for such specification, or whether additional forces are necessary. Regarding the topographic targeting along the D-V axis, consistent with the continuous nature at the coarse level, several molecules, including transcription factors and cell-surface receptors, are expressed as gradients along this axis (Norlin et al., 2001). A recent study provided functional evidence that Slit-1 and Robo-2, a classic ligand-receptor pair for repulsive axon guidance, are required for axons from ORNs located in dorsomedial OE (expressing high levels of Robo-2) to avoid ventral OB (expressing high levels of Slit-1) (Cho et al., 2007). It seems likely that Slit-1/Robo-2, perhaps in combination with other ligand-receptor pairs that are distributed in a graded fashion along the D-V axis, might provide the instructive forces to establish the coarse topographic targeting along this axis.

Recent advances implicating a second step of refinement after the initial coarse targeting make the daunting task of specifying discrete addresses for 1000 ORN classes more manageable. Sensory experience and neuronal activity have been suggested to play a role in the maturation and refinement of ORN axon targeting (e.g., Zhao and Reed, 2001; Yu et al., 2004; Zou et al., 2004). Alteration in the levels of ephrin-A proteins, classic axon guidance molecules involved in the formation of visual maps (section 2), resulted in subtle changes of glomerular targeting position (Cutforth et al., 2003). A recent study found a connection between the above phenomena: levels of ephrin-A5, and its receptor EphA5, are regulated by sensory activity. Whereas EphA5 transcription is upregulated by activity, ephrin-A5 is downregulated (Serizawa et al., 2006). Moreover, different OR classes express different levels of ephrin-A (Cutforth et al., 2003; Serizawa et al., 2006) and EphA5 (Serizawa et al., 2006), so that neighboring glomeruli exhibit a complementary expression pattern of this pair of repulsive guidance molecules. Interestingly, the levels of another pair of Ig-domain-containing homophilic adhesion molecules, Kirrel2 and Kirrel3, in ORNs also correlate with OR classes and are also up- and downregulated by activity, respectively. Furthermore, overexpressing Kirrel2 in half of ORNs of a specific class causes the target glomerulus to split into two adjacent glomeruli (Serizawa et al., 2006). Thus, the OR-correlated and activity-dependent expression of homophilic adhesion proteins and repulsive or bifunctional ligand-receptor pairs can be used to ensure that axons belonging to the same OR find each other and segregate from axons belonging to other ORN classes near their target glomeruli (Figure 4C). It will be interesting in the future to quantitatively analyze the degree of local glomerular segregation afforded by Kirrel2/3, ephrin-A5/EphA5 and other such protein pairs acting alone or as a combinatorial code and test whether such local segregation in combination with global forces (such as cAMP levels in the A-P axis) comes close to explaining the precise wiring of all ORN classes.

3.3 Mechanisms of ORN Axon Targeting in Flies

Despite the striking similarity in the organization of their olfactory systems (Hildebrand and Shepherd, 1997), an important difference between flies and mice is that ORs do not participate in ORN axon targeting in flies (Dobritsa et al., 2003; Wang et al., 2003). Indeed, the expression onset of most OR genes is after targeting is completed. The participation of ORs in targeting may be a vertebrate invention to accommodate the large increase in OR genes and glomerular number. Identifying mechanisms that allow fly ORNs to find their targets may uncover evolutionarily ancient mechanisms that work together with, or are co-opted by, OR-dependent mechanisms in mice.

When pioneering ORN axons enter the brain, the developing antennal lobe is already prepatterned by PN dendrites. Different PN classes have already sent their dendrites to the approximate area corresponding to their future glomerular targets (Jefferis et al., 2004; see below). Thus, ORN axon targeting can (1) use global cues in the antennal lobe, (2) sort each other out by axon-axon

interactions similar to what has been postulated above for the mouse, (3) use cues located on their future synaptic partner PN dendrites.

Convincing global cues for ORN axon targeting have not been reported, but the following examples suggest their usage. First, when the cell adhesion molecule N-cadherin was removed from ORNs, their axons remain at the antennal lobe surface without invading into the antennal lobe to form proper synaptic contact with PN dendrites. Consequently, glomeruli do not form. Yet *N-cadherin* mutant ORNs of specific classes still target their axons to the surface areas of the antennal lobe that roughly correspond to the positions of their glomerular targets (Hummel and Zipursky, 2004). These findings suggest that global targeting does not require interactions with PN dendrites or the formation of glomeruli. Second, as discussed earlier, pairs or multiple ORNs belonging to different classes reside in the same sensillum but target their axons to different glomeruli. When Notch signaling is disrupted, such differences disappear, and ORNs target to glomeruli belonging to "Notch-OFF" ORN classes within the same sensillum. Notch-OFF and Notch-ON glomeruli have a stereotyped organization in the antennal lobe (Endo et al., 2007) (Figure 5C). The simplest interpretation is that Notch signaling diversifies ORN cell types by conferring them with expression of different guidance receptors, which allow their axons to target to distinct areas in response to the same global cues in the antennal lobe. This study also provided strong evidence that coordination of ORN axon targeting and OR expression later is constrained by their lineages, upon which Notch signaling acts to diversify cell types from the same lineage (Endo et al., 2007).

The involvement of axon-axon interactions in ORN axon targeting can be inferred from studies analyzing mutants in the cell adhesion molecule Dscam and transcription factor Acj6. Mutant axons of the same ORN class often form clusters in ectopic places, implying self-recognition and/or stabilization of ORN axons that belong to the same class (Hummel et al., 2003; Komiyama et al., 2004). Furthermore, in mosaic animals, wild-type ORN axons mistarget when other ORNs are mutant for Acj6, suggesting that hierarchical interactions among different ORN classes are necessary for axon targeting of certain ORN classes (Komiyama et al., 2004). The transmembrane axon guidance molecule Sema-1a and its receptor plexinA have recently been identified to play a role in such axon-axon interactions (Lattemann et al., 2007; Sweeney et al., 2007). Deprivation of Sema-1a from early-arriving axons from antennal ORNs causes late-arriving wild-type maxillary palp axons to mistarget to areas normally occupied by antennal ORN axons, suggesting that Sema-1a made by antennal axons acts as a repulsive ligand to constrain target choice of maxillary palp axons through direct axon-axon interactions at the target (Sweeney et al., 2007) (Figure 5D). This strategy of temporal target restriction through axon-axon interactions may be used in the development of other neural maps, including the retinotopic map (see section 2.4).

The existence of cues on PN dendrites that are used for ORN axon targeting was inferred from a serendipitous finding. Overexpressing Dscam in PNs that target dendrites to adjacent glomeruli frequently causes one of these glomeruli (VA1d) to swap position with a neighboring glomerulus (VA1lm). When this happens, ORN axons that normally target to VA1d or VA1lm always follow the mispositioned PN dendrites, rather than targeting to their normal spatial locations (Zhu et al., 2006a). This observation implies that a direct recognition of PN dendrites by ORN axons acts at least at the final step to ensure correct synaptic matching. It will be interesting to test whether this is generally applicable to other ORN classes, how far PN dendrites can be mispositioned and still achieve correct synaptic matching, and what molecules mediate such synaptic matching.

In summary, existing evidence supports a multistep targeting process whereby the identities of ORNs are determined by a combination of their lineage and spatial location in the peripheral sensory organs. This identity confers on them expression of odorant receptors and specific guidance molecules that allow each ORN class to uniquely respond to global cues in the antennal lobe, cues from axons of other ORN classes and from their partner PN dendrites.

3.4 Mechanisms of PN Dendrite Targeting in Flies

A special feature of the fly olfactory map, not previously described in any other neural circuits, is the active and precise dendrite targeting of second-order olfactory projection neurons (PNs). (For recent examples of dendrite targeting in vertebrates, see Mumm et al., 2006; Vrieseling and Arber, 2006.) At the transition between larva and pupa, PNs start to elaborate their dendrites. Within the next 18 hr or so, these dendrite elaborations create the proto-antennal lobe ready for ORN axons to innervate. Remarkably, each of the ~50 classes of PNs have sorted out their dendrites such that they occupy a small portion of the proto-antennal lobe corresponding to their future glomerular positions (Jefferis et al., 2004) (Figure 5D).

Clonal analysis suggested that PN dendrite targeting utilizes information from neuroblast lineage and birth order (Jefferis et al., 2001) (Figure 5B). Indeed, candidate transcription factors that carry lineage and birth-order information have been identified. Two POU-domain transcription factors, Acj6 and Drifter, are expressed specifically in two major PN lineages, and loss- and gain-of-function experiments suggest that they play an instructive role in specifying PN dendrite targeting appropriate for their lineages (Komiyama et al., 2003). On the other hand, the zinc-finger transcription factor Chinmo is expressed in multiple neuroblast lineages, but Chinmo protein forms a temporal gradient within each lineage such that neurons born earlier from the same lineage have more Chinmo proteins than those born later. When *chinmo* is deleted from early-born PNs, their dendrites are targeted to glomeruli appropriate for their younger sisters (Zhu et al., 2006b). Despite these advances, we are still at the beginning of identifying the entire transcription factor code for PN dendrite targeting (Komiyama and Luo, 2007).

Transcription factor codes are presumably turned into cell-surface receptor codes that allow PN dendrites of different classes to respond differentially to the same environment. So far, only one cell-surface protein, Sema-1a, has been shown to play an instructive role in PN dendrite targeting. Interestingly, unlike in ORNs where Sema-1a acts cell-nonautonomously as a ligand in axon-axon interactions (section 3.3), Sema-1a acts cell-autonomously as a receptor to direct PN dendrite targeting. Furthermore, different PN classes express different amounts of Sema-1a in their dendrites, such that Sema-1a protein forms a gradient along the dorsolateral to ventromedial axis. Loss- and gain-of-function experiments support a model in which levels of Sema-1a instruct PN dendrite targeting along this axis (Komiyama et al., 2007). Future experiments identifying the ligand for Sema-1a, and other ligand-receptor pairs that instruct PN dendrite targeting along this and other axes, will provide a more complete picture of how the coarse dendrite map is generated.

The coarse dendrite map is refined into a discrete glomerular map after the arrival of ORN axons. The mechanism by which this is achieved is unknown, but likely involves complex cellular interactions. In addition to the ORN-PN axon-dendrite and ORN axon-axon interactions described above, dendrite-dendrite interactions among PNs have also been shown to be essential to refine dendrites of individual PNs within one glomerulus (Zhu and Luo, 2004). A future challenge is to determine how these forces act concertedly to help establishing the final glomerular map and how discreteness emerges.

4. Discussion

On the surface, olfactory and retinotopic projections appear to be very different types of map, one toward the discrete extreme and the other at the continuous extreme. Can common themes nevertheless be discerned in the mechanisms used to construct them? If so, we can expect similar principles to be used in other maps throughout the nervous system.

One common theme in mapping is the existence of one set of mechanisms to establish an initial rough map, followed by another set for map refinement. Gradients of labels are well established as a mechanism to set up initial order in continuous topographic maps. While the role of gradients of Ephs and ephrins has been especially well studied in retinotopy, it also extends to many other continuous maps. Interestingly, in olfactory maps, the use of graded signaling systems and graded labels is also now emerging as a developmental strategy. Why use gradients in mapping? Gradients have two important advantages. First, they can be highly economical, since a small number of graded molecules can specify many positional values across an entire developmental field. Second, gradients can provide information not only about final position, but also about direction. Rather than axons having to search the target by a random walk, gradients can be sensed at distant points in the target and can be used by axons to navigate toward their destination.

Gradients have the limitation, however, that they are an inherently imprecise way to reliably distinguish nearby points. Refinement mechanisms can increase the precision. In retinotopic mapping, refinement is achieved by an instructive role for correlated neural activity. In the olfactory system, sharp discrimination can be achieved by glomerulus-specific labels such as levels of Kirrels and ephrins, which can segregate axons in a discrete manner to the correct glomerulus. These seemingly different refinement strategies used in vision and olfaction have similarities and might reflect similar underlying mechanisms. In particular, expression of the glomerulus-specific labels is found to be dependent on neural activity. In the future, it will be interesting to know whether the role for activity in olfactory mapping is instructive, using correlated activity in a Hebbian-type mechanism, whether it is odorant-evoked or spontaneous, and whether visual map refinement may employ the same molecular segregation mechanisms that distinguish adjacent glomeruli.

Another common theme is the use of axon-axon interactions to ensure filling of the target in an orderly manner. An apogee of this principle is seen in the fly retinotopic projections, where individual axons target to a unique cartridge or column. In this case, axon-axon interactions mediated by cell-surface molecules in the cadherin and IgCAM families can determine the orderly guidance and tiling of axons to form a stereotypic array in the target. In the vertebrate retinotectal projection, axon-axon interactions ensure that the entire target is filled with axons smoothly and completely. While the molecular mechanisms for this are still not well understood, the requirement of an IgCAM for smooth target filling is intriguing, since it suggests that target filling in vertebrates and axon tiling in flies may reflect the same underlying principle of mapping.

Axon-axon interactions are also used extensively in both vertebrate and fly olfactory maps. In the mouse, adhesive and repulsive axon-axon interactions are likely used for local sorting of axon terminals correlating with ORN identity. In the fly, repulsive axon-axon interactions at the target are used to ensure that different ORN classes occupy different areas of the antennal lobe.

In addition to common principles, a comparison of mapping strategies can reveal features specific to particular types of map. For example, where patterning information resides and how maps are sequentially assembled appear to differ for different neural maps. The development of the fly visual system is driven by the input field and involves a spatiotemporal mechanism of local interactions where retinal units develop sequentially and can induce the differentiation and patterning of their corresponding target units. While this mechanism seems especially well suited to the highly ordered matching arrays found in the fly visual system, similar spatiotemporal principles could be used in other maps. Patterning information in the vertebrate retinotopic map and fly olfactory map resides in both input and target fields. In the case of the fly olfactory map, patterning of PN dendrite precedes the arrival of ORN axons.

In general, independent patterning allows target neurons to coordinate their input with their own output as in the case of fly olfactory system. Future studies will clarify to what extent these differences reflect our partial understanding or reflect differences in size, developmental, functional, and evolutionary constraints of each neural map.

Retinotopic and olfactory glomerular maps represent two ends of a continuum that includes many other types of neural map in between. We predict that the emerging principles outlined above will be used in the development of many neural maps described in this issue and in additional neural maps yet to be discovered in the nervous system.

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