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Axon–axon interactions in neuronal circuit assembly: lessons from olfactory map formation

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Abstract

During the development of the nervous system, neurons often connect axons and dendrites over long distances, which are navigated by chemical cues. During the past few decades, studies on axon guidance have focused on chemical cues provided by the axonal target or intermediate target. However, recent studies have shed light on the roles and mechanisms underlying axon–axon interactions during neuronal circuit assembly. The roles of axon–axon interactions are best exemplified in recent studies on olfactory map formation in vertebrates. Pioneer–follower interaction is essential for the axonal pathfinding process. Pre-target axon sorting establishes the anterior–posterior map order. The temporal order of axonal projection is converted to dorsal–ventral topography with the aid of secreted molecules provided by early-arriving axons. An activity-dependent process to form a discrete map also depends on axon sorting. Thus, an emerging principle of olfactory map formation is the 'self-organisation' of axons rather than the 'lock and key' matching between axons and targets. In this review, we discuss how axon–axon interactions contribute to neuronal circuit assembly.

Introduction

Axon–target vs. axon–axon interactions

Over 100 years ago, Ramón y Cajal first described the axonal growth cone and assumed that this structure may be guided by chemical cues (Ramón y Cajal, 1892; de Castro *et al.*, 2007). This idea was experimentally supported by Roger Sperry using eye rotation experiments and a series of regeneration experiments in the retinotectal system, which led to the proposal of the 'chemoaffinity hypothesis' (Sperry, 1963). Since then, the retinotectal system has motivated many neurobiologists to study the molecular basis of axon guidance.

In the retinotectal system, retinal ganglion cells project axons in a topographical fashion, such that the nasal–temporal and dorsal–ventral orders in the retina are maintained in the tectum along the posterior–anterior and ventral–dorsal axes, respectively. Thus, the external world screened on the retina is spatially represented in the tectum, which is referred to as a visual map or, more generally, a neural map. Neural maps are also referred to in other sensory systems (e.g. somatosensory, auditory and olfactory systems), and are believed to serve as an important platform for extracting and processing meaningful features from sensory stimuli.

Sperry proposed that the axons of retinal ganglion cells are guided by graded chemical tags presented by the tectum or along the pathway. The molecular mechanisms of axon–target interactions have since been a major issue in axon guidance research. Many neurobiologists have investigated the molecular nature of the chemical tags, and the first such molecules identified for visual map formation were ephrin-A ligands and EphA receptors (Cheng *et al.*, 1995; Drescher *et al.*, 1995). Ephrin-A ligands are expressed in an anterior-low and posterior-high gradient in the tectum or a mammalian equivalent, the superior colliculus (SC). In contrast, EphA receptors are expressed in the retina, forming the nasal-low and temporal-high gradient. Ephrin-A ligands repel temporal retinal axons expressing EphA. A dozen molecules are known to be expressed in a graded manner in the retina and/or tectum (SC) and function in visual map formation (reviewed by Flanagan, 2006; Feldheim & O'Leary, 2010). It is thought that repulsive and attractive actions of tectum (SC)-derived factors navigate retinal axons and thereby establish a topographic visual map.

However, these guidance molecules do not seem to determine the absolute positions of axonal projection sites. A half ablation of the tectum results in a compression of the visual map into the remaining half of the tectum (Yoon, 1976). Conversely, when the retina is half ablated, the remaining retinal axons expand to cover the entire tectal region (Yoon, 1977). More recently, it was shown that overexpression of the EphA3 receptor in a mosaic population of retinal ganglion cells resulted in duplicated visual maps in the SC, one for the original EphA levels and another for elevated EphA levels (Brown *et al.*, 2000). Thus, relative but not absolute expression levels of guidance receptors determine axonal projection sites (Reber *et al.*, 2004). The underlying mechanisms of this axonal competition remain unclear.

The study of the olfactory map has a relatively short history, because it began only after the discovery of odorant receptors (ORs; Buck & Axel, 1991). In the rodent main olfactory system, odorants are detected by ~1000 types of olfactory sensory neurons (OSNs), each expressing a single type of OR (Malnic *et al.*, 1999; Serizawa *et al.*,

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2003). OSNs expressing a given type of OR converge their axons to a pair of glomeruli in stereotypical locations of the olfactory bulb (OB), one in the medial and another in the lateral map (Mombaerts *et al.*, 1996). Thus, the receptor-topic olfactory map is formed in the OB. Odour information is spatially represented in the OB as the activation pattern of glomeruli (reviewed by Mori *et al.*, 2006). Because the OR protein itself is a determinant of axonal projection sites, many researchers have assumed that ORs or their associated guidance receptors might detect positional cues presented by the OB (Wang *et al.*, 1998; Cutforth *et al.*, 2003). However, every effort to identify such positional cues in the OB has been unsuccessful. Instead, growing evidence suggests a model in which axon–axon interactions self-organise the olfactory map. An extreme situation demonstrating this idea was the OB-less *Gli3* mutant mouse; even in the absence of the OB, OSNs expressing the same OR converged (St John *et al.*, 2003), maintaining the gross map order (Imai *et al.*, 2009).

Recent studies have indicated that multiple aspects of OSN projection depend on axon–axon interactions in the vertebrate olfactory system. We review the developmental process of the vertebrate olfactory map formation and discuss how axon–axon interactions contribute to neuronal circuit assembly in general. For a more comprehensive review on the olfactory map, see Mori & Sakano (2011).

Pioneer–follower interactions determine axonal trajectory

The axon guidance process is often initiated by the 'pioneer' population of neurons (Bate, 1976; Ho & Goodman, 1982; Raper *et al.*, 1983; reviewed by Raper & Mason, 2010). In some systems, ablation of pioneer neurons perturbs or delays the guidance of follower neurons (Edwards *et al.*, 1981; Raper *et al.*, 1984; Klose & Bentley, 1989). Thus, the axons of pioneer neurons are assumed to serve as important scaffolds for follower axons.

In the zebrafish olfactory system, axonal projection is initiated by a transient population of neurons, i.e. pioneer neurons (Fig. 1). Pioneer neurons are morphologically and histochemically distinct from OSNs and do not appear to express ORs (Whitlock & Westerfield, 1998). Laser ablation of pioneer neurons prevented the normal projection of OSNs; OSN axons typically extended posteriorly toward the diencephalon (Whitlock & Westerfield, 1998). Some axon guidance molecules are known to regulate the axonal projection of pioneer olfactory neurons. Robo2 is transiently expressed in the olfactory placode in zebrafish (Miyasaka *et al.*, 2005). In *Robo2* mutant zebrafish, pioneer neurons were misrouted and often overshot the OB. OSN axons are also misrouted posteriorly toward the diencephalon, reminiscent of the phenotype of pioneer neuron ablation. Similarly, *Cxcr4b* mutant zebrafish also show defects in targeting pioneer axons (Miyasaka *et al.*, 2007). Mice may also utilise similar mechanisms in the initial phase of OSN projection. In *Robo1/Robo2* double-knockout mice, OSN axons were often misguided ventrally to the medial septum and failed to innervate the OB (Nguyen-Ba-Charvet *et al.*, 2008), consistent with the zebrafish study.

A recent investigation in the zebrafish visual system sought to determine whether pioneer axons were sufficient to guide follower axons. When development of pioneer neurons was blocked by *ath5* morpholino knockdown, retinal axons failed to exit the eye. However, this phenotype was rescued by transplanting non-morphant pioneer neurons. It has been demonstrated that *Robo2*-deficient zebrafish show mistargeting of retinal axons (Hutson & Chien, 2002). When *Robo2* mutant pioneer neurons were transplanted to *ath5* morphants, wild-type follower axons also mistargeted (Pittman *et al.*, 2008). Conversely, when wild-type pioneer neurons were transplanted to *ath5* morphant *Robo2* mutant fish, follower axons lacking Robo2 projected well, almost like wild-type axons (Pittman *et al.*, 2008). These findings indicate that the guidance of follower axons largely depends on the trajectories of pioneer axons.

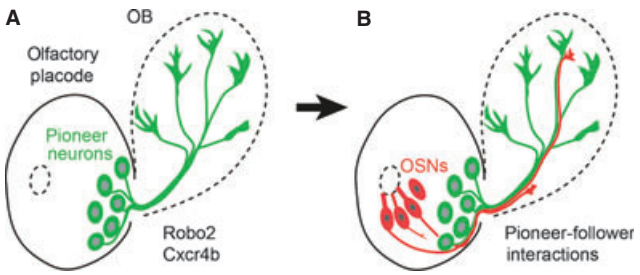

FIG. 1. Pioneer–follower interaction in the olfactory axon guidance. (A) In the zebrafish olfactory system, axonal projection is initiated by pioneer neurons (green), which are unipolar in shape and histochemically distinct from olfactory sensory neurons (OSNs). (B) Bipolar OSNs (orange) extend axons along the trajectories of pioneer axons. Laser ablation of pioneer neurons results in mistargeting of following OSN axons, suggesting that pioneer axons serve as cues or scaffolding for following OSN axons. Robo2 and Cxcr4b are required for pioneer axon projection. The molecular mechanisms underlying pioneer–follower interaction remain unknown. OB, olfactory bulb.

Patterning of heterotypic axons also depends on axon–axon interaction. Fasciculation and projection patterns of sensory and motor axons in the mouse limb are highly correlated, and both are regulated by Neuropilin-1 (Nrp1; Huber *et al.*, 2005). Notably, sensory neuron-specific knockout of *Nrp1* impaired axonal fasciculation not only in sensory neurons but also in motor neurons (Huettl *et al.*, 2011). Thus, sensory axons serve as scaffolds for motor axon projection. The molecular mechanisms underlying the pioneer–follower interaction remain poorly understood.

Selective axonal fasciculation in preventing miswiring in the pathway

The segregation of heterotypic axons is also essential in neuronal circuit assembly. In the mouse olfactory system, OSN axons projecting to main OBs and vomeronasal sensory neuron axons projecting to the accessory OB are selectively fasciculated, forming distinct axon bundles. In *Sema3F* mutant mice, vomeronasal axons expressing Neuropilin-2 (Nrp2) were defasciculated and occasionally invaded the main OB (Cloutier *et al.*, 2002).

Selective axonal fasciculation has been thoroughly studied for sensory and motor axons in the mouse axial limb. As mentioned above, fasciculation and targeting of sensory and motor axons are often highly coordinated. However, these two types of axons are also segregated into mutually exclusive internerve fascicules. In the axial nerves, this heterotypic axonal segregation is regulated by EphAs expressed by motor axons and ephrin-As expressed by sensory axons. In EphA3/EphA4 double-mutant mice, motor axons are missorted to sensory axon fascicules (Gallarda *et al.*, 2008). Thus, trans-axonal ephrin-A-to-EphA forward signalling establishes discrete peripheral afferent and efferent pathways.

Pre-target axon sorting establishes the anterior–posterior axis of the olfactory map

In the mouse olfactory system, OR protein plays an instructive role in OSN projection. When a coding sequence of an OR was swapped for

that of another one, the OSN projection sites shifted to distinct locations along the anterior–posterior axis of the OB (Mombaerts *et al.*, 1996; Wang *et al.*, 1998; Feinstein & Mombaerts, 2004). Furthermore, OR protein was detected not only in the olfactory cilia but also at OSN axon terminals (Barnea *et al.*, 2004; Strotmann *et al.*, 2004). Based on these findings, many researchers assumed that OR protein itself might act as an axon guidance receptor. However, later studies have argued against this model.

It has been reported that OR-derived cAMP signals are essential for OSN projection (reviewed by Imai & Sakano, 2007). When a conserved DRY motif of an OR, essential for G-protein coupling, was mutated, OSN axons mistargeted the anterior OB, failing to form a convergent glomerular structure. This defective phenotype was rescued by co-expressing a constitutively active mutant of Gs, PKA or CREB (Imai *et al.*, 2006). Furthermore, genetic manipulation to increase or decrease the level of cAMP signals caused posterior and anterior shift of projection sites, respectively (Imai *et al.*, 2006; Chesler *et al.*, 2007; Dal Col *et al.*, 2007; Zou *et al.*, 2007). This anterior–posterior shift of OSN projection sites is mediated by transcriptional regulation of a set of axon guidance molecules, including Nrp1 (Imai *et al.*, 2006). The expression level of *Nrp1* is positively regulated by OR-derived cAMP signals. A gain-of-function or loss-of-function of *Nrp1* in OSNs expressing a particular OR caused posterior and anterior shifts of glomeruli, respectively (Imai *et al.*, 2009). Mice deficient in Sema3A, a repulsive secreted ligand for Nrp1, and OSN-specific Nrp1 knockout mice showed a distorted olfactory map, forming multiple ectopic glomeruli along the anterior–posterior axis (Schwarting *et al.*, 2000; Imai *et al.*, 2009).

How do Nrp1 and Sema3A establish the anterior–posterior map order? In the mouse olfactory system, target neurons seem to play minimal roles in OSN projection. A normal olfactory map was established in the absence of projection neurons (in *Tbr-1*-deficient mice) or γ-aminobutyric acid (GABA)ergic interneurons (in *Dlx-1*/Dlx-2-deficient mice; Bulfone *et al.*, 1998). Furthermore, convergence of OSNs expressing the same OR and anterior–posterior axis formation were both preserved, even in the complete absence of the OB in *Gli3* mutant mice (St John *et al.*, 2003; Imai *et al.*, 2009). Consistent with these findings, axons projecting to distinct destinations in the OB were presorted in the axon bundle (Satoda *et al.*, 1995; Bozza *et al.*, 2009; Imai *et al.*, 2009).

Nrp1 and *Sema3A* are expressed in OSNs in a complementary manner. Within the axon bundle projecting to the dorsolateral OB (Fig. 2A), Nrp1-expressing OSN axons are sorted to the outer lateral region, whereas Sema3A-expressing OSN axons are sorted to the central region. Gain-of-function or loss-of-function of *Nrp1* in a subset of OSNs changed the axon trajectories. Furthermore, OSN-specific knockout of *Sema3A* perturbed both pre-target axon sorting and glomerular positioning (Imai *et al.*, 2009). Thus, Nrp1-expressing axons are repelled by Sema3A-expressing axons and thereby project to the posterior region of the OB.

However, OSN axon-derived Sema3A alone would be insufficient to explain correct map orientation. *Sema3A* is transiently expressed in the anterior OB and ensheathing glial cells along the medial side of the axon bundle. OSN axon sorting and olfactory map formation were more severely disrupted in *Sema3A* total-knockout mice than in OSN-specific mice (Schwarting *et al.*, 2000; Taniguchi *et al.*, 2003; Imai *et al.*, 2009). Thus, it is conceivable that Sema3A expressed by the target and intermediate target direct the pre-sorted axons along a correct axis of the map. It has been proposed that axon–axon interactions establish the relative positions of axons, and that target-derived cues serve as 'directional cues' rather than positional cues (Imai *et al.*, 2009; Fig. 2B).

As mentioned above, visual map topography is also determined by relative expression levels of axon guidance receptors. Further-

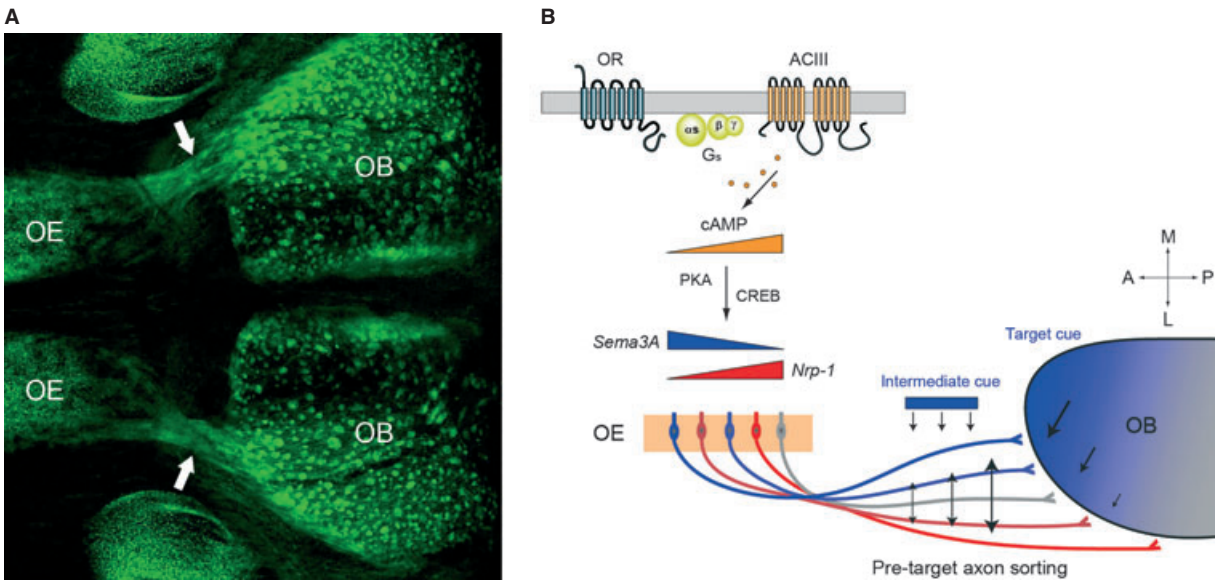

FIG. 2. Pre-target axon sorting establishes the anterior–posterior axis of the olfactory map. (A) Visualisation of the OSN projection from the olfactory epithelium (OE) to the olfactory bulb (OB). A fixed whole-mount sample of OMP-GFP mice of age P7 was optically cleared, and fluorescent images were taken with a confocal microscope (dorsal view). A stacked confocal image spanning 480 μm thickness is shown. Axon bundles projecting to the dorsolateral OBs are indicated by arrows. OSNs in the OE are fasciculated to form several axon bundles as they extend. Once OSN axons enter the OB, they become defasciculated and cover the OB surface. An anterior–posterior map order emerges in the axon bundles. (B) The mechanisms underlying anterior–posterior axis formation. Each odorant receptor (OR) is thought to generate a unique level of cAMP signals, which subsequently regulate the expression of axon guidance molecules; *Nrp1* and *Sema3A* are positively and negatively regulated by cAMP signals, respectively. OSN-derived Sema3A repels Nrp1-expressing OSN axons leading to pre-target sorting of axons. Once OSN axons are sorted, they must be oriented along the correct axis of the OB. This probably requires intermediate and target cues. A dorsolateral axon bundle and a horizontal OB section (dorsal view) are schematically shown. Distinct axon bundles (not shown) target the medial OB. A, anterior; ACIII, adenylyl cyclase type III; L, lateral; M, medial; P, posterior.

more, pre-target axon sorting has been described in the visual system, particularly in fish and frogs (Scholes, 1979). Because topographic axon sorting emerges only after axons cross the optic chiasm, pre-target axon sorting is not just a passive consequence. In chickens and mice, anterior–posterior mapping in the SC occurs largely on the target through axon branching; however, dorsal–ventral mapping is already evident in the axon tract (Plas *et al.*, 2005; reviewed by Feldheim & O'Leary, 2010). Furthermore, axon–axon repulsion has been demonstrated for retinal axons *in vitro* (Bonhoeffer & Huf, 1985; Raper & Grunewald, 1990). Because ephrins and Ephs are both expressed in the retina, ephrins/Ephs may act not only between axons and targets but also among axons in the axon bundle and/or on the surface of the target. Alternatively, pre-target axon sorting may be regulated by distinct mechanisms (Karlstrom *et al.*, 1996). In the zebrafish visual system, maternally supplied *ext2* and *extl3*, essential for heparan sulphate synthesis, are required for pre-target axon sorting and topographic axonal projection (Lee *et al.*, 2004).

Pattern formation by sorting seems to be a general and important strategy in development. Although morphogenesis is often thought to occur based on the morphogen concentration, it seems to be very difficult for cells to detect the 'absolute' concentration. In contrast, cells can easily detect the relative concentration of extracellular ligands; for example, leucocytes can detect a ~2% difference in chemoattractant concentrations between the front and back of the cell surface (Wang, 2009). It has been reported that pattern formation in the multicellular state of *Dictyostelium* amoebae occurs through random differentiation in a salt and pepper fashion and subsequent cell sorting without a morphogen gradient (Kay & Thompson, 2009). Cell sorting may occur through differential cell motility or be based on differential cell adhesion (Steinberg & Takeichi, 1994).

The temporal sequence of OSN projection is converted to the dorsal–ventral topographic order

In contrast to the anterior–posterior OSN projection, the dorsal–ventral axis of the map is correlated with the location of OSNs in the olfactory epithelium (OE); the dorsal–ventral positioning of glomeruli is correlated with the dorsomedial–ventrolateral expression zone of the OR (Astic *et al.*, 1987; Miyamichi *et al.*, 2005). When an OR was expressed in an ectopic zone of the OE, ectopic glomeruli were formed in a distinct domain of the OB (Miyamichi *et al.*, 2005). Robo2 and Nrp2, expressed in a graded manner along the dorsomedial–ventrolateral axis in the OE, are essential for topographic dorsal–ventral OSN projection.

During the embryonic development, OSNs in the dorsomedial zone project earlier than ventrolateral OSNs (Sullivan *et al.*, 1995; Takeuchi *et al.*, 2010). Robo2 is expressed in a dorsomedial-high and ventrolateral-low fashion, and required for the topographic projection of dorsomedial OSN axons (Cho *et al.*, 2007, 2011; Nguyen-Ba-Charvet *et al.*, 2008). Slit1, a repulsive ligand for Robo2, is expressed in the septum and ventral OB during the early embryonic stage, and thus appears to restrict early-arriving dorsomedial OSN axons to the dorsal part of the OB (Fig. 3A).

How are late-arriving ventrolateral OSN axons then arranged in the ventral OB? Topographic projection of ventrolateral OSNs depends on Nrp2 (Takahashi *et al.*, 2010; Takeuchi *et al.*, 2010). In the OE, *Nrp2* is expressed forming a dorsomedial-low and ventrolateral-high gradient (Norlin *et al.*, 2001). Gain-of-function and loss-of-function of *Nrp2* in a subset of OSNs caused the ventral and dorsal shift of glomeruli, respectively, suggesting that the expression levels of Nrp2 determine dorsal–ventral positioning of glomeruli (Takeuchi *et al.*, 2010). However, *Sema3F* mRNA, coding for a repulsive secreted ligand for Nrp2, was not expressed in the OB. Instead, *Sema3F* mRNA was expressed only in the dorsomedial OSNs. However, EYFP-tagged Sema3F protein was found in the outer olfactory nerve layer of the dorsal OB, suggesting that Sema3F is secreted from axon terminals of dorsomedial OSNs. OSN-specific knockout of *Sema3F* perturbed the dorsal–ventral axis of the olfactory map (Takeuchi *et al.*, 2010). Taken together, these results indicate that topographic projection of late-arriving ventrolateral OSN axons is regulated by Sema3F supplied by early-arriving dorsomedial OSN axons (Fig. 3B).

Similarly, early-arriving antennal OSN axons express repulsive ligand Sema1A in the *Drosophila* olfactory system. In contrast, late-arriving maxillary pulp OSN axons express Plxn-A, a receptor for Sema1A. Sema1A expressed by early-arriving axons is required to restrict the targeting area of late-arriving axons (Sweeney *et al.*, 2007). Because the temporal order of neurogenesis, migration and maturation is known to be an important determinant of neuronal identity and circuit assembly in the brain (reviewed by Pearson & Doe, 2004),

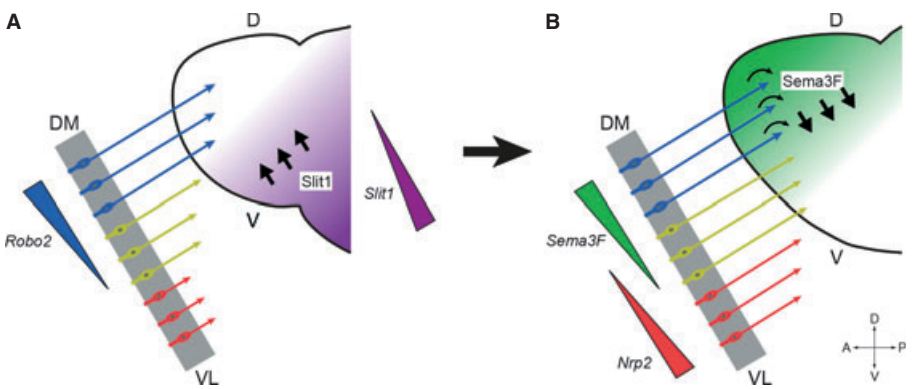

FIG. 3. The sequential projection of OSN axons results in the dorsal–ventral axis of the map. (A) In the OE, OSNs in the dorsomedial zone mature earlier and project axons to the OB earlier than ventrolateral OSNs. Dorsomedial OSNs express an axon guidance receptor, Robo2. Slit1, a repulsive ligand for Robo2, is expressed in the septum and ventral OB during early development. As a result, dorsomedial OSNs project axons to the prospective dorsal domain of the embryonic OB. (B) Ventrolateral OSNs express an axon guidance receptor, Nrp2. Its repulsive ligand, Sema3F, is expressed in dorsomedial OSNs, but not in the OB. The axonal projection of OSNs occurs sequentially along the dorsomedial–ventrolateral axis of the OE as the OB grows ventrally during development. Sema3F secreted by dorsomedial OSN axons in the OB prevents late-arriving $Nrp2^+$ axons from invading the dorsal region of the OB. This may help maintain the topographic order between the OE and OB during axonal projection. Medial sides of the OBs are schematically shown. A, anterior; D, dorsal; DM, dorsomedial; P, posterior; V, ventral; VL, ventrolateral.

interactions between early- and late-arriving axons may be a general strategy for neuronal wiring.

Activity-dependent local axon sorting in forming a discrete olfactory map

A combination of anterior–posterior patterning based on OR-derived cAMP signals and dorsal–ventral patterning based on the OSN positional information generates the coarse topography of the olfactory map. However, the hallmark of the olfactory map in the OB is the discrete map, where each discrete glomerulus receives inputs from homogeneous OSN axons expressing the same OR. Further refinement to form the discrete map requires activity-dependent processes at later developmental stages. Developmental studies have shown that neighbouring glomeruli are intermingled before birth, and discrete glomeruli emerge after early neonatal stages (Conzelmann *et al.*, 2001; Potter *et al.*, 2001; Sengoku *et al.*, 2001). CNGA2 mutant mice, which lack most odour-evoked neuronal activity, show a larger number of ectopic glomeruli in the OB (Zheng *et al.*, 2000). Overexpression of inward rectifying potassium channel Kir2.1 in all OSNs resulted in reduced spontaneous activity and poor axonal convergence (Yu *et al.*, 2004). In the visual map formation, spontaneous activity, known as 'retinal wave', instructs map refinement through the Hebbian mechanism, i.e. based on synchronised activation of postsynaptic neurons (reviewed by Firth *et al.*, 2005). However, such a mechanism does not seem to explain the activity-dependent process of olfactory map formation; expression of a tetanus toxin light chain in all OSNs, which blocks the synaptic transmission from OSN axon terminals, did not cause defects in glomerular segregation (Yu *et al.*, 2004).

It has been shown that neuronal activity in OSNs regulates several genes coding for adhesion and sorting molecules, and thereby facilitates local axon sorting (Serizawa *et al.*, 2006; Fig. 4). Kirrel2 and Kirrel3 are homophilic cell adhesion molecules containing five immunoglobulin domains. Neuronal activity regulates Kirrel2 and Kirrel3 positively and negatively, respectively. As a result, these two genes are expressed in a complementary manner in OSNs. Gain-of-function of Kirrel2 or Kirrel3 in a mosaic population of neurons produced duplicated glomeruli for an OR, one for normal Kirrel2/3 levels and another for overexpressed Kirrel2/3 levels. Thus, it is proposed that activity-high and activity-low axons are selectively fasciculated by Kirrel2/3-mediated adhesion (Serizawa *et al.*, 2006). In contrast to Kirrel2/3, EphA and ephrin-A are thought to segregate heterotypic axons through their repulsive actions; EphA and ephrin-A are positively and negatively regulated by neuronal activity, respectively (Serizawa *et al.*, 2006). BIG2, which is regulated in an OR-specific fashion, is also thought to facilitate local axon sorting with a currently unidentified heterophilic binding partner (Kaneko-Goto *et al.*, 2008). Other candidate molecules involved in local axon sorting include OL-protocadherin (Aoki *et al.*, 2003) and the protocadherin-alpha family (Hasegawa *et al.*, 2008).

These findings are consistent with earlier studies demonstrating the role of axon–axon interactions in axonal convergence. The successful convergence of homotypic OSN axons was dependent on OSN number (Ebrahimi & Chess, 2000). Extensive OR mutagenesis changed the sorting behaviour of axons (Feinstein & Mombaerts, 2004).

Maintenance of the map during regeneration

In the mouse olfactory system, OSNs are continuously renewed and replaced throughout life, with a life span ranging from 30 to 120 days

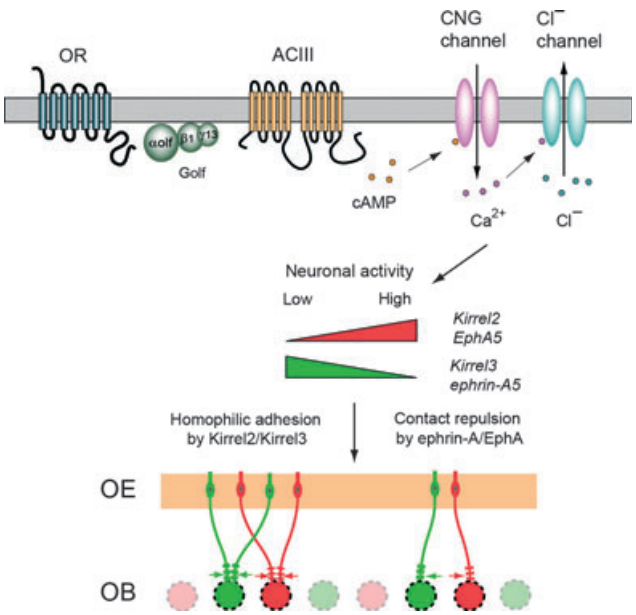

FIG. 4. Activity-dependent axon sorting in forming the discrete map. Although odorant receptor (OR)/cAMP signals regulate the coarse targeting of OSN axons along the anterior–posterior axis at an earlier stage of patterning, neuronal activity generated by OR/cAMP signals regulates local axon sorting at a later stage. This may indicate that cAMP signals are generated by different mechanisms in earlier and later stages (reviewed by Imai & Sakano, 2008). Levels of neuronal activity determine different sets of axon guidance and adhesion molecules in different manners. These include Kirrel2/Kirrel3, ephrin-A/EphA and BIG2. Kirrel2/Kirrel3 mediates the local fasciculation of homotypic axons via homophilic adhesion activities, whereas ephrin-A/EphA is thought to segregate heterotypic axons via contact repulsion. BIG2 is involved in heterophilic adhesion. ACIII, adenylyl cyclase type III; CNG channel, cyclic nucleotide-gated channel; OB, olfactory bulb; OE, olfactory epithelium.

(reviewed by Astic & Saucier, 2001). How is the initial olfactory map then maintained throughout life over many rounds of regeneration?

It has been shown that even when ~95% of OSNs expressing OR P2 were specifically ablated with diphtheria toxin during the developmental stage or in adults, normal axonal convergence was restored as OSNs were renewed (Gogos *et al.*, 2000). However, when all OSN axons were killed by axotomy (Costanzo, 2000; Christensen *et al.*, 2001) or by administering the chemical toxin dichlobenil (St John & Key, 2003), olfactory map topography and OR-specific convergence were not restored in the regenerated map. A possible explanation for this discrepancy is that existing axons serve as cues or scaffolding for the projection of renewing OSN axons. The molecular mechanisms underlying the axon–axon interactions that maintain the olfactory map during the regeneration process remain unclear, and identifying these mechanisms will be an important topic of research for future studies. In humans, severe virus infection or trauma can result in anosmia or parosmia even after the recovery period (Temmel *et al.*, 2002). This type of olfactory dysfunction is probably due to the distorted olfactory map topography after regeneration. Thus, understanding the regeneration process is important from a therapeutic standpoint.

Concluding remarks

As discussed in this review, many aspects of olfactory map formation depend on axon–axon interactions. Roles of axon–axon interactions have also been studied in other model systems, such as *Drosophila* photoreceptor neuron projection, where axon–axon interactions are

important for target selection, regular axon spacing and tiling (Clandinin & Zipursky, 2000; Millard *et al.*, 2007; Chen & Clandinin, 2008; Tomasi *et al.*, 2008; reviewed by Schwabe *et al.*, 2009). Although Sperry's chemoaffinity hypothesis and subsequent studies have mostly focused on interactions between axons and their targets (or intermediate targets; reviewed by Dickson, 2002; Kolodkin & Tessier-Lavigne, 2011), growing evidence suggests that axon–axon interaction is also an important aspect of neuronal wiring. Neuronal circuit formation does not solely depend on axon–target interactions utilising the lock and key mechanism. Rather, neuronal circuits are self-assembled, at least partly, based on interactions among axons. This idea provides important insight into the evolution of the brain, because this mechanism would need fewer guidance molecules and would thus allow easier addition of new neuronal circuits to the brain during the evolutionary process. Furthermore, our understanding of axon–axon interaction might provide insight into the regenerative medicine in the future. Recent progress in stem cell research has succeeded in generating peripheral and central neuron subtypes from pluripotent stem cells *in vitro*. However, the next major obstacle is integrating these neurons into pre-existing circuits. The self-assembling properties of neurons might provide insight that will help to overcome this problem in the future.

In the olfactory system, our knowledge of neuronal wiring mechanisms is still largely limited to peripheral neurons. Connections between the OB and olfactory cortex are just beginning to be elucidated at a single glomerulus and single axon resolution (Nagayama *et al.*, 2010; Ghosh *et al.*, 2011; Miyamichi *et al.*, 2011; Sosulski *et al.*, 2011). Although the peripheral map is formed in an autonomous fashion, the behavioural output is sometimes OR-specific, stereotyped and robust (Lin *et al.*, 2005; Kobayakawa *et al.*, 2007; Chamero *et al.*, 2007; reviewed by Zufall & Leinders-Zufall, 2007). The next major challenge is to understand the wiring mechanisms underlying OB circuits and cortical projection. How does each mitral/tufted cell innervate a single glomerulus? Is this connection deterministic or stochastic? What rules determine the seemingly complex connection of interneurons and centrifugal fibres in the OB? What rules and mechanisms underlie cortical projection by mitral/tufted cells? These questions should be addressed in future studies.

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Abbreviations

Nrp1, neuropilin-1; Nrp2, neuropilin-2; OB, olfactory bulb; OE, olfactory epithelium; OR, odorant receptor; OSN, olfactory sensory neuron; SC, superior colliculus.

References

Aoki, E., Kimura, R., Suzuki, S.T. & Hirano, S. (2003) Distribution of OL-protocadherin protein in correlation with specific neural compartments and local circuits in the postnatal mouse brain. *Neuroscience*, **117**, 593–614.

Astic, L. & Saucier, D. (2001) Neuronal plasticity and regeneration in the olfactory system of mammals: morphological and functional recovery following olfactory bulb deafferentation. *Cell. Mol. Life Sci.*, **58**, 538–545.

Astic, L., Saucier, D. & Holley, A. (1987) Topographical relationships between olfactory receptor cells and glomerular foci in the rat olfactory bulb. *Brain Res.*, **424**, 144–152.

Barnea, G., O'Donnell, S., Mancia, F., Sun, X., Nemes, A., Mendelsohn, M. & Axel, R. (2004) Odorant receptors on axon termini in the brain. *Science*, **304**, 1468–1468.

Bate, C.M. (1976) Pioneer neurones in an insect embryo. *Nature*, **260**, 54–56.

Bonhoeffer, F. & Huf, J. (1985) Position-dependent properties of retinal axons and their growth cones. *Nature*, **315**, 408–410.

Bozza, T., Vassalli, A., Fuss, S., Zhang, J.J., 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.

Brown, A., Yates, P.A., Burrola, P., Ortuno, D., Vaidya, A., Jessell, T.M., Pfaff, S.L., O'Leary, D.D.M. & 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.

Buck, L. & Axel, R. (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell*, **65**, 175–187.

Bulfone, A., Wang, F., Hevner, R., Anderson, S., Cutforth, T., Chen, S., Meneses, J., Pedersen, R., Axel, R. & Rubenstein, J.L.R. (1998) An olfactory sensory map develops in the absence of normal projection neurons or GABAergic interneurons. *Neuron*, **21**, 1273–1282.

de Castro, F., Lopez-Mascaraque, L. & De Carlos, J.A. (2007) Cajal: lessons on brain development. *Brain Res. Rev.*, **55**, 481–489.

Chamero, P., Marton, T.F., Logan, D.W., Flanagan, K., Cruz, J.R., Saghatelian, A., Cravatt, B.F. & Stowers, L. (2007) Identification of protein pheromones that promote aggressive behaviour. *Nature*, **450**, 899–U823.

Chen, P.L. & Clandinin, T.R. (2008) The cadherin flamingo mediates level-dependent interactions that guide photoreceptor target choice in Drosophila. *Neuron*, **58**, 26–33.

Cheng, H.J., Nakamoto, M., Bergemann, A.D. & Flanagan, J.G. (1995) Complementary gradients in expression and binding of ELF-1 and Mek4 in development of the topographic retinotectal projection map. *Cell*, **82**, 371–381.

Chesler, A.T., Zou, D.J., Le Pichon, C.E., Peterlin, Z.A., Matthews, G.A., Pei, X., Miller, M.C. & Firestein, S. (2007) A G protein/cAMP signal cascade is required for axonal convergence into olfactory glomeruli. *Proc. Natl. Acad. Sci. USA*, **104**, 1039–1044.

Cho, J.H., Lepine, M., Andrews, W., Parnavelas, J. & Cloutier, J.F. (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.

Cho, J.H., Prince, J.E., Cutforth, T. & Cloutier, J.F. (2011) The pattern of glomerular map formation defines responsiveness to aversive odorants in mice. *J. Neurosci.*, **31**, 7920–7926.

Christensen, M.D., Holbrook, E.H., Costanzo, R.M. & Schwob, J.E. (2001) Rhinotopy is disrupted during the re-innervation of the olfactory bulb that follows transection of the olfactory nerve. *Chem. Senses*, **26**, 359–369.

Clandinin, T.R. & Zipursky, S.L. (2000) Afferent growth cone interactions control synaptic specificity in the Drosophila visual system. *Neuron*, **28**, 427–436.

Cloutier, J.F., Giger, R.J., Koentges, G., Dulac, C., Kolodkin, A.L. & Ginty, D.D. (2002) Neuropilin-2 mediates axonal fasciculation, zonal segregation, but not axonal convergence, of primary accessory olfactory neurons. *Neuron*, **33**, 877–892.

Conzelmann, S., Malun, D., Breer, H. & Strotmann, J. (2001) Brain targeting and glomerulus formation of two olfactory neuron populations expressing related receptor types. *Eur. J. Neurosci.*, **14**, 1623–1632.

Costanzo, R.M. (2000) Rewiring the olfactory bulb: changes in odor maps following recovery from nerve transection. *Chem. Senses*, **25**, 199–205.

Cutforth, T., Moring, L., Mendelsohn, M., Nemes, A., Shah, N.M., Kim, M.M., Frisen, J. & Axel, R. (2003) Axonal ephrin-as and odorant receptors: Coordinate determination of the olfactory sensory map. *Cell*, **114**, 311–322.

Dal Col, J.A., Matsuo, T., Storm, D.R. & Rodriguez, I. (2007) Adenylyl cyclase-dependent axonal targeting in the olfactory system. *Development*, **134**, 2481–2489.

Dickson, B.J. (2002) Molecular mechanisms of axon guidance. *Science*, **298**, 1959–1964.

Drescher, U., Kremoser, C., Handwerker, C., Loschinger, J., Noda, M. & Bonhoeffer, F. (1995) In vitro guidance of retinal ganglion cell axons by RAGS, a 25 kDa tectal proteinrelated to ligands for Eph receptor tyrosine kinases. *Cell*, **82**, 359–370.

Ebrahimi, F.A.W. & Chess, A. (2000) Olfactory neurons are interdependent in maintaining axonal projections. *Curr. Biol.*, **10**, 219–222.

Edwards, J.S., Chen, S.W. & Berns, M.W. (1981) Cercal sensory development following laser microlesions of embryonic apical cells in Acheta domesticus. *J. Neurosci.*, **1**, 250–258.
Feinstein, P. & Mombaerts, P. (2004) A contextual model for axonal sorting into glomeruli in the mouse olfactory system. *Cell*, **117**, 817–831.
Feldheim, D.A. & O'Leary, D.D.M. (2010) Visual map development: bidirectional signaling, bifunctional guidance molecules, and competition. *Cold Spring Harb. Perspect. Biol.*, **2**, a001768.
Firth, S.I., Wang, C.T. & Feller, M.B. (2005) Retinal waves: mechanisms and function in visual system development. *Cell Calcium*, **37**, 425–432.
Flanagan, J.G. (2006) Neural map specification by gradients. *Curr. Opin. Neurobiol.*, **16**, 59–66.
Gallarda, B.W., Bonanomi, D., Muller, D., Brown, A., Alaynick, W.A., Andrews, S.E., Lemke, G., Pfaff, S.L. & Marquardt, T. (2008) Segregation of axial motor and sensory pathways via heterotypic trans-axonal signaling. *Science*, **320**, 233–236.
Ghosh, S., Larson, S.D., Hefzi, H., Marnoy, Z., Cutforth, T., Dokka, K. & Baldwin, K.K. (2011) Sensory maps in the olfactory cortex defined by long-range viral tracing of single neurons. *Nature*, **472**, 217–220.
Gogos, J.A., Osborne, J., Nemes, A., Mendelsohn, M. & Axel, R. (2000) Genetic ablation and restoration of the olfactory topographic map. *Cell*, **103**, 609–620.
Hasegawa, S., Hamada, S., Kumode, Y., Esumi, S., Katori, S., Fukuda, E., Uchiyama, Y., Hirabayashi, T., Mombaerts, P. & Yagi, T. (2008) The protocadherin-alpha family is involved in axonal coalescence of olfactory sensory neurons into glomeruli of the olfactory bulb in mouse. *Mol. Cell. Neurosci.*, **38**, 66–79.
Ho, R.K. & Goodman, C.S. (1982) Peripheral pathways are pioneered by an array of central and peripheral neurones in grasshopper embryos. *Nature*, **297**, 404–406.
Huber, A.B., Kania, A., Tran, T.S., Gu, C.H., Garcia, N.D., Lieberam, I., Johnson, D., Jessell, T.M., Ginty, D.D. & Kolodkin, A.L. (2005) Distinct roles for secreted semaphorin signaling in spinal motor axon guidance. *Neuron*, **48**, 949–964.
Huettl, R.E., Soellner, H., Bianchi, E., Novitch, B.G. & Huber, A.B. (2011) Npn-1 contributes to axon-axon interactions that differentially control sensory and motor innervation of the limb. *PLoS Biol.*, **9**, e1001020.
Hutson, L.D. & Chien, C.B. (2002) Pathfinding and error correction by retinal axons: the role of astray/robo2. *Neuron*, **33**, 205–217.
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.
Kaneko-Goto, T., Yoshihara, S., Miyazaki, H. & Yoshihara, Y. (2008) BIG-2 mediates olfactory axon convergence to target glomeruli. *Neuron*, **57**, 834–846.
Karlstrom, R.O., Trowe, T., Klostermann, S., Baier, H., Brand, M., Crawford, A.D., Grunewald, B., Haffter, P., Hoffmann, H., Meyer, S.U., Muller, B.K., Richter, S., vanEeden, F.J.M., NusseinVolhard, C. & Bonhoeffer, F. (1996) Zebrafish mutations affecting retinotectal axon pathfinding. *Development*, **123**, 427–438.
Kay, R.R. & Thompson, C.R.L. (2009) Forming patterns in development without morphogen gradients: scattered differentiation and sorting out. *Cold Spring Harb. Perspect. Biol.*, **1**, a001503.
Klose, M. & Bentley, D. (1989) Transient pioneer neurons are essential for formation of an embryonic peripheral nerve. *Science*, **245**, 982–984.
Kobayakawa, K., Kobayakawa, R., Matsumoto, H., Oka, Y., Imai, T., Ikawa, M., Okabe, M., Ikeda, T., Itohara, S., Kikusui, T., Mori, K. & Sakano, H. (2007) Innate versus learned odour processing in the mouse olfactory bulb. *Nature*, **450**, 503–U505.
Kolodkin, A.L. & Tessier-Lavigne, M. (2011) Mechanisms and molecules of neuronal wiring: a primer. *Cold Spring Harb. Perspect. Biol.*, **3**, a001727.
Lee, J.S., von der Hardt, S., Rusch, M.A., Stringer, S.E., Stickney, H.L., Talbot, W.S., Geisler, R., Nusslein-Volhard, C., Selleck, S.B., Chien, C.B. & Roehl, H. (2004) Axon sorting in the optic tract requires HSPG synthesis by ext2 (dackel) and extl3 (boxer). *Neuron*, **44**, 947–960.
Lin, D.Y., Zhang, S.Z., Block, E. & Katz, L.C. (2005) Encoding social signals in the mouse main olfactory bulb. *Nature*, **434**, 470–477.
Malnic, B., Hirono, J., Sato, T. & Buck, L.B. (1999) Combinatorial receptor codes for odors. *Cell*, **96**, 713–723.
Millard, S.S., Flanagan, J.J., Pappu, K.S., Wu, W. & Zipursky, S.L. (2007) Dscam2 mediates axonal tiling in the Drosophila visual system. *Nature*, **447**, 720–724.
Miyamichi, K., Serizawa, S., Kimura, H.M. & 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.
Miyamichi, K., Amat, F., Moussavi, F., Wang, C., Wickersham, I., Wall, N.R., Taniguchi, H., Tasic, B., Huang, Z.J., He, Z.G., Callaway, E.M., Horowitz, M.A. & Luo, L.Q. (2011) Cortical representations of olfactory input by trans-synaptic tracing. *Nature*, **472**, 191–196.
Miyasaka, N., Sato, Y., Yeo, S.Y., Hutson, L.D., Chien, C.B., Okamoto, H. & Yoshihara, Y. (2005) Robo2 is required for establishment of a precise glomerular map in the zebrafish olfactory system. *Development*, **132**, 1283–1293.
Miyasaka, N., Knaut, H. & Yoshihara, Y. (2007) Cxcl12/Cxcr4 chemokine signaling is required for placode assembly and sensory axon pathfinding in the zebrafish olfactory system. *Development*, **134**, 2459–2468.
Mombaerts, P., Wang, F., Dulac, C., Chao, S.K., Nemes, A., Mendelsohn, M., Edmondson, J. & Axel, R. (1996) Visualizing an olfactory sensory map. *Cell*, **87**, 675–686.
Mori, K. & Sakano, H. (2011) How is the olfactory map formed and interpreted in the mammalian brain? *Annu. Rev. Neurosci.*., **34**, 467–499.
Mori, K., Takahashi, Y.K., Igarashi, K.M. & Yamaguchi, M. (2006) Maps of odorant molecular features in the mammalian olfactory bulb. *Physiol. Rev.*, **86**, 409–433.
Nagayama, S., Enerva, A., Fletcher, M.L., Masurkar, A.V., Igarashi, K.M., Mori, K. & Chen, W.R. (2010) Differential axonal projection of mitral and tufted cells in the mouse main olfactory system. *Front. Neural Circuits*, **4**, 120.
Nguyen-Ba-Charvet, K.T., Di Meglio, T., Fouquet, C. & Chédotal, A. (2008) Robos and slits control the pathfinding and targeting of mouse olfactory sensory axons. *J. Neurosci.*, **28**, 4244–4249.
Norlin, E.M., 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.
Pearson, B.J. & Doe, C.Q. (2004) Specification of temporal identity in the developing nervous system. *Annu. Rev. Cell Dev. Biol.*, **20**, 619–647.
Pittman, A.J., Law, M.Y. & Chien, C.B. (2008) Pathfinding in a large vertebrate axon tract: isotypic interactions guide retinotectal axons at multiple choice points. *Development*, **135**, 2865–2871.
Plas, D.T., Lopez, J.E. & Crair, M.C. (2005) Pretarget sorting of retinocollicular axons in the mouse. *J. Comp. Neurol.*, **491**, 305–319.
Potter, S.M., Zheng, C., Koos, D.S., Feinstein, P., Fraser, S.E. & Mombaerts, P. (2001) Structure and emergence of specific olfactory glomeruli in the mouse. *J. Neurosci.*, **21**, 9713–9723.
Ramón y Cajal, S. (1892) La rétine des vertébrés. *La Cellule*, **9**, 121–255.
Raper, J.A. & Grunewald, E.B. (1990) Temporal retinal growth cones collapse on contact with nasal retinal axons. *Exp. Neurol.*, **109**, 70–74.
Raper, J. & Mason, C. (2010) Cellular strategies of axonal pathfinding. *Cold Spring Harb. Perspect. Biol.*, **2**, a001933.
Raper, J.A., Bastiani, M. & Goodman, C.S. (1983) Pathfinding by neuronal growth cones in grasshopper embryos. II. Selective fasciculation onto specific axonal pathways. *J. Neurosci.*, **3**, 31–41.
Raper, J.A., Bastiani, M.J. & Goodman, C.S. (1984) Pathfinding by neuronal growth cones in grasshopper embryos. IV. The effects of ablating the A and P axons upon the behavior of the G growth cone. *J. Neurosci.*, **4**, 2329–2345.
Reber, M., Burrola, P. & Lemke, G. (2004) A relative signalling model for the formation of a topographic neural map. *Nature*, **431**, 847–853.
Satoda, M., Takagi, S., Ohta, K., Hirata, T. & Fujisawa, H. (1995) Differential expression of two cell surface proteins, neuropilin and plexin, in Xenopus olfactory axon subclasses. *J. Neurosci.*, **15**, 942–955.
Scholes, J.H. (1979) Nerve fibre topography in the retinal projection to the tectum. *Nature*, **278**, 620–624.
Schwabe, T., Gontang, A.C. & Clandinin, T.R. (2009) More than just glue: the diverse roles of cell adhesion molecules in the Drosophila nervous system. *Cell Adh. Migr.*, **3**, 36–42.
Schwarting, G.A., Kostek, C., Ahmad, N., Dibble, C., Pays, L. & Puschel, A.W. (2000) Semaphorin 3A is required for guidance of olfactory axons in mice. *J. Neurosci.*, **20**, 7691–7697.
Sengoku, S., Ishii, T., Serizawa, S., Nakatani, H., Nagawa, F., Tsuboi, A. & Sakano, H. (2001) Axonal projection of olfactory sensory neurons during the developmental and regeneration processes. *Neuroreport*, **12**, 1061–1066.

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.

Sosulski, D.L., Bloom, M.L., Cutforth, T., Axel, R. & Datta, S.R. (2011) Distinct representations of olfactory information in different cortical centres. *Nature*, **472**, 213–216.

Sperry, R.W. (1963) Chemoaffinity in orderly growth of nerve fiber patterns and connections. *Proc. Natl. Acad. Sci. USA*, **50**, 703–710.

St John, J.A. & Key, B. (2003) Axon mis-targeting in the olfactory bulb during regeneration of olfactory neuroepithelium. *Chem. Senses*, **28**, 773–779.

St John, J.A., Clarris, H.J., 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.

Steinberg, M.S. & Takeichi, M. (1994) Experimental specification of cell sorting, tissue spreading, and specific spatial patterning by quantitative differences in cadherin expression. *Proc. Natl. Acad. Sci. USA*, **91**, 206–209.

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.

Sullivan, S.L., Bohm, S., Ressler, K.J., Horowitz, L.F. & Buck, L.B. (1995) Target-independent pattern specification in the olfactory epithelium. *Neuron*, **15**, 779–789.

Sweeney, L.B., Couto, A., Chou, Y.H., Berdnik, D., Dickson, B.J., Luo, L.Q. & Komiyama, T. (2007) Temporal target restriction of olfactory receptor neurons by Semaphorin-1a/PlexinA-mediated axon-axon interactions. *Neuron*, **53**, 185–200.

Takahashi, H., Yoshihara, S., Nishizumi, H. & Tsuboi, A. (2010) Neuropilin-2 is required for the proper targeting of ventral glomeruli in the mouse olfactory bulb. *Mol. Cell. Neurosci.*, **44**, 233–245.

Takeuchi, H., Inokuchi, K., Aoki, M., Suto, F., Tsuboi, A., Matsuda, I., Suzuki, M., Aiba, A., Serizawa, S., Yoshihara, Y., Fujisawa, H. & Sakano, H. (2010) Sequential arrival and graded secretion of Sema3F by olfactory neuron axons specify map topography at the bulb. *Cell*, **141**, 1056–1067.

Taniguchi, M., Nagao, H., Takahashi, Y.K., 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.

Temmel, A.F., Quint, C., Schickinger-Fischer, B., Klimek, L., Stoller, E. & Hummel, T. (2002) Characteristics of olfactory disorders in relation to major causes of olfactory loss. *Arch. Otolaryngol. Head Neck Surg.*, **128**, 635–641.

Tomasi, T., Hakeda-Suzuki, S., Ohler, S., Schleiffer, A. & Suzuki, T. (2008) The transmembrane protein golden goal regulates R8 photoreceptor axon-axon and axon-target interactions. *Neuron*, **57**, 691–704.

Wang, F. (2009) The signaling mechanisms underlying cell polarity and chemotaxis. *Cold Spring Harb. Perspect. Biol.*, **1**, a002980.

Wang, F., Nemes, A., Mendelsohn, M. & Axel, R. (1998) Odorant receptors govern the formation of a precise topographic map. *Cell*, **93**, 47–60.

Whitlock, K.E. & Westerfield, M. (1998) A transient population of neurons pioneers the olfactory pathway in the zebrafish. *J. Neurosci.*, **18**, 8919–8927.

Yoon, M.G. (1976) Progress of topographic regulation of the visual projection in the halved optic tectum of adult goldfish. *J. Physiol.*, **257**, 621–643.

Yoon, M.G. (1977) Induction of compression in the re-established visual projections on to a rotated tectal reimplant that retains its original topographic polarity within the halved optic tectum of adult goldfish. *J. Physiol.*, **264**, 379–410.

Yu, C.R., Power, J., Barnea, G., O'Donnell, S., Brown, H.E., Osborne, J., Axel, R. & Gogos, J.A. (2004) Spontaneous neural activity is required for the establishment and maintenance of the olfactory sensory map. *Neuron*, **42**, 553–566.

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.

Zou, D.J., Chesler, A.T., Le Pichon, C.E., Kuznetsov, A., Pei, X., Hwang, E.L. & Firestein, S. (2007) Absence of adenylyl cyclase 3 perturbs peripheral olfactory projections in mice. *J. Neurosci.*, **27**, 6675–6683.

Zufall, F. & Leinders-Zufall, T. (2007) Mammalian pheromone sensing. *Curr. Opin. Neurobiol.*, **17**, 483–489.