Chapter 2
Semaphorin Regulation of Neural Circuit Assembly in the Central Nervous System

Randal A. Hand and Alex L. Kolodkin

Abstract The assembly of neural circuits requires a wide array of molecular cues. These cues include secreted and transmembrane ligands and also the signaling receptors that together modulate axonal and dendritic morphology to promote functional neural connectivity during development. The semaphorin family of proteins and their various receptors serve this function. Many experiments demonstrate in vivo, and in multiple neural systems, how semaphorin-mediated regulation of neuronal morphology is critical for the regulation of neuronal connectivity. This system is nicely illustrated by recent work on semaphorin function in establishing neural connections in the olfactory and visual systems in both flies and mice. Further, semaphorins and their receptors regulate the elaboration of axon trajectories and precise targeting of these projections in the mammalian central nervous system, in addition to mediating axon pruning and also excitatory and inhibitory synaptogenesis. Taken together, these investigations into how semaphorins regulate neural connectivity provide insight into developmental mechanisms critical for neurite targeting, laminar-specific innervation, selective synapse formation, and neural circuit refinement.

Keywords Neural circuits • Semaphorin • Neural development • Plexin • Axon guidance • Dendritic morphology • Visual system • Olfactory system • Hippocampus • Cerebral cortex

2.1 Introduction

The establishment of a central nervous system during neural development requires a diverse and complex array of neurons to be selectively assembled into functioning neural circuits. This assembly depends upon neurons precisely responding to environmental cues to form neural circuits, both locally within a given structure and distantly among neurons residing in distinct structures. A wide range of

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neuronal guidance cues and their receptors mediate neuronal migrations, influence the elaboration of distinct neuronal morphologies, guide axons and dendrites to their appropriate targets, and also regulate synaptogenesis. Semaphorins, a large group of extracellular signaling molecules, are essential for the assembly of neural circuits in both invertebrates and vertebrates. Many studies show that semaphorins are essential for mediating short- and long-range axon guidance, sculpting dendritic morphology, facilitating synapse formation, and directing axon pruning. Here, we explore recent findings that demonstrate roles for semaphorin signaling during neural circuit formation and refinement in the central nervous system.

2.2 Semaphorins Direct Circuit Assembly in the Developing Visual System

A fully functional visual system requires precise wiring within the highly stratified neural retina (Fig. 2.1a) and also accurate central projections by retinal ganglion cells (RGCs) to appropriate central nervous system targets. Recent studies demonstrate the importance of semaphorins and their receptors for the development of neural circuits in the vertebrate and invertebrate visual systems. Here, we consider these new findings, which advance our understanding of laminar organization within the retina and of the specificity of central projections from the eye to central visual system targets in the brain.

Membrane-bound semaphorins have recently been identified as key regulators of neuronal process stratification in both vertebrate and invertebrate visual systems. The *Drosophila melanogaster* visual system is composed of several interconnected neuronal structures. The fly retina contains eight photoreceptor neuronal subtypes (R1–R8) that send axonal projections to either the lamina (R1–R6) or the medulla (R7 and R8), the first- and second-order neuropils, respectively, that initially process visual information in this system. Lamina neurons (L1–L5), in turn, form stereotypic connections with neuronal targets in the medulla (Fig. 2.1b) (Sanes and Zipursky 2010). Several recent studies show that the transmembrane semaphorin Sema-1a is a key regulator of laminar-specific connectivity within the *Drosophila* visual system.

In Sema-1a mutant flies, R1–R6 photoreceptor (PR) cell axons fail to fasciculate in a tight bundle and stray from their normal target area in the lamina. This defect in *Sema-1a* mutants can be rescued by providing wild-type Sema-1a to mutant PRs; however, Sema-1a lacking its cytoplasmic domain fails to rescue Sema-1a mutant PR axon-targeting defects, suggesting that Sema-1a functions here not as a ligand but as a receptor, so-called "reverse signaling," to regulate the stereotypic R1–R6 PR lamina innervation (Cafferty et al. 2006). In support of this finding, mutations in the *Sema-1a* receptor gene, *PlexA*, phenocopy PR axonal-targeting defects observed in *Sema-1a* mutants, and in gain-of-function (GOF) experiments full-length PlexA and also PlexA lacking its cytoplasmic domain are functionally equivalent (Yu et al. 2010). Taken together, these results suggest PlexA serves as a ligand for Sema-1a reverse signaling, a function also observed in embryonic *Drosophila* motor neurons (Jeong et al. 2012).

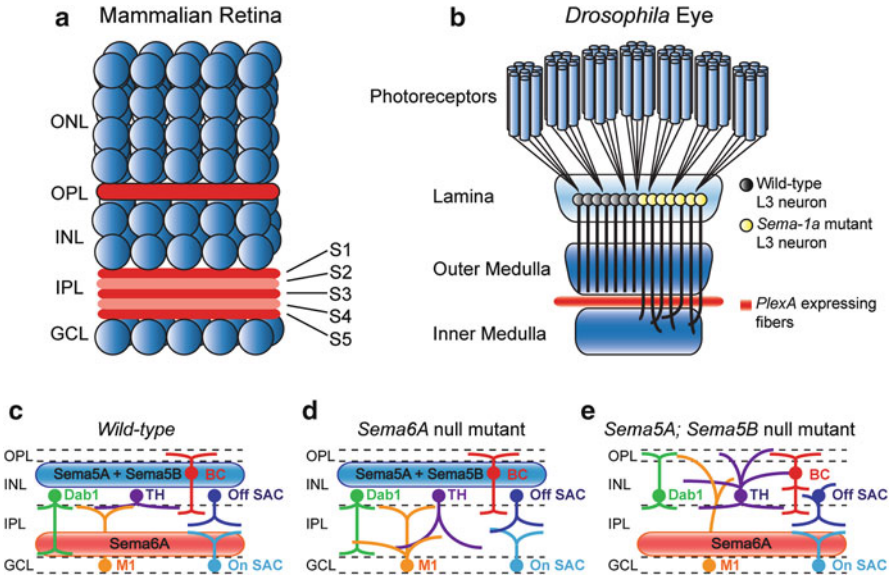

Fig. 2.1 Schematic diagrams of the mammalian retina and *Drosophila* visual system. (**a**) The mammalian retina consists of three nuclear layers (*blue*) containing neuronal cell bodies and two plexiform layers containing their neurites (*red*). (**b**) The *Drosophila* visual system includes photoreceptors that send axons to the lamina and the medulla. Lamina neurons (*grey circles*) have axons that innervate the medulla. In the Sema-1a null mutant, L3 neuron (*yellow circles*) axons overshoot their normal laminar targets in the outer medulla and extend into the inner medulla. PlexA-expressing fibers located between the outer and inner medulla serve to repel L3 axons through Sema-1a reverse signaling. (**c**) A diagram of Sema5A, Sema5B, and Sema6A expression patterns in the mouse retina, and the wild-type innervation patterns of retinal neurons: Dab1-positive amacrine cell (*Dab1*); TH-positive amacrine cell (*TH*); M1-type melanopsin intrinsically photosensitive RGC (*M1*); bipolar cells (BP); Off starburst amacrine cell (*Off SAC*); and On starburst amacrine cell (*On SAC*). (**d**) In Sema6A-null mutants, the sublaminar targeting in the IPL is disrupted in Dab1- and TH-positive amacrine cells, M1-type melanopsin intrinsically photosensitive RGCs, Off SACs, and On SACs. (**e**) Sema5A and Sema5B serve to repel neurites from the INL. In Sema5A–Sema5B double-null mutants, Dab1- and TH-positive amacrine cells and M1-type melanopsin intrinsically photosensitive RGCs and bipolar cells, and ON SACs aberrantly innervate the INL

In addition to regulating *Drosophila* PR axon guidance, Sema-1a plays a critical role in targeting axons from lamina (L) neurons to select strata within the medulla (Pecot et al. 2013). L3 neurons selectively innervate the M3 layer of the outer medulla within the developing *Drosophila* visual system. Interestingly, Sema-1a localizes to growth cones of L3 neuron axons. Following either RNAi-mediated knockdown of Sema-1a in L neurons, or single-cell genetic ablation of Sema-1a in L3 neurons using the MARCM (mosaic analysis with a repressible cell marker) technique, L3 axons project beyond the M3 layer and into the inner medulla (Fig. 2.1b). The outer region of the inner medulla contains fibers that express high levels of PlexA, and ubiquitous RNAi-mediated knockdown of PlexA results in the

mistargeting of L3 neuron axons into the inner medulla. Interestingly, these results again suggest that Sema-1a reverse signaling is important for the precise wiring of the *Drosophila* visual system, and this signaling apparently is repulsive. Taken together, these results show that transmembrane semaphorin reverse signaling is necessary for the assembly of multiple neural circuits within the invertebrate visual system.

Transmembrane semaphorins are also essential for establishing connectivity within the mammalian visual system. The mammalian retina is organized into a highly stratified structure that includes several nuclear layers: the outer nuclear layer (ONL), composed of rod and cone photoreceptor cell bodies; the inner nuclear layer (INL), composed of cell bodies belonging to bipolar cells, horizontal cells, and amacrine cells; and the ganglion cell layer (GCL), which includes cell bodies of retinal ganglion cells (RGCs) and displaced amacrine cells. Between these nuclear layers exist plexiform layers: dense networks of neurites and synapses that serve to interconnect neurons whose cell bodies reside in the nuclear layers. The outer plexiform layer (OPL) is located between the ONL and the INL and is where photoreceptors, bipolar cells, and horizontal cell establish contacts with one another. The inner plexiform layer (IPL) is located between the INL and the GCL, contains multiple sublamina (S1–S5, S5 being located closest to the GCL), and serves to interconnect neurites extending from bipolar cells, amacrine cells, and RGCs (Fig. 2.1a) (Sanes and Zipursky 2010). RGCs are the projection neurons of the retina, and their axons extend into the central nervous system.

Transmembrane semaphorins Sema5A, Sema5B, and Sema6A are essential for establishing neural circuit formation within the plexiform layers of the mammalian retina (Fig. 2.1c–e). Sema5A and Sema5B are both expressed within the INL, where they serve to organize and constrain neurites that normally stratify within the IPL from extending into outer retina regions (Matsuoka et al. 2011a). In *Sema5A*; *Sema5B* double-null mutants, neurites from amacrine cells, bipolar cells, and RGCs fail to target in appropriate IPL sublamina and instead extend toward the INL and into the INL. Some of these neurites even extended all the way into the OPL. Type 1 and 2 Off cone-bipolar cells normally innervate both the OPL and IPL, but in *Sema5A*; *Sema5B* double mutants bipolar cell neurites were also observed innervating the INL. Sema5A and Sema5B in the developing retina serve as ligands for the PlexA1 and PlexA3 receptors, and *PlexA1*; *PlexA3* double-null mutants phenocopy the neurite targeting errors observed in *Sema5A*; *Sema5B* double-null mutants (Matsuoka et al. 2011a). Therefore, transmembrane Sema5 proteins play a general role in establishing correct neurite arborization in IPL sublaminae through their ability to constrain neurites to this plexiform layer. But how is specific laminar targeting by neurites from individual neuronal subtypes in the retina achieved?

In the developing chick retina, GOF and LOF experiments show that adhesion molecules belonging to the immunoglobulin (IG) superfamily provide short-range cues important for specific stratification events among BPs, RGCs, and ACs that co-stratify within the IPL (Sanes and Yamagata 2009). Recent studies demonstrate the importance of repulsive guidance in regulating laminar-specific targeting and retinal neuron morphology, showing that the transmembrane semaphorin Sema6A

and its receptors, PlexA2 and PlexA4, serve key roles in establishing the sublaminar organization of the IPL and regulating the arborization of horizontal cell and On starburst amacrine cell (SAC) neurites. Reminiscent of Sema-1a and PlexA expression patterns in the outer and inner medulla of the *Drosophila* eye (Pecot et al. 2013), Sema6A is highly expressed in the IPL of the mouse retina in neurites within sublaminae S4 and S5, whereas PlexA4 is highly expressed in a small subset of neurites that stratify their projections in sublaminae S1 and S2. In either *Sema6A* or *PlexA4* null mutants, neurites from calbindin-positive amacrine cells, dopaminergic amacrine cells, and M1-type melanopsin intrinsically photosensitive RGCs (ipRGCs) innervate inappropriate sublaminae within the IPL, consistent with Sema6A serving to constrain a small subset of amacrine cell and RGC neurites to laminar targets in the outer IPL (Matsuoka et al. 2011b). Sema6A and PlexA4 protein expression is complementary, even very early in the development of IPL stratification (for example, at postnatal day 2), suggesting that the initial establishment of repulsive IPL regions sets the stage for specific laminar targeting observed later in retinal development. Sema6A also regulates the sublaminar targeting of SAC neurites in the IPL (Sun et al. 2013). Interestingly, Off SAC neurites innervate the S2 IPL lamina and express PlexA2, whereas On SAC neurites innervate S4 and express both Sema6A and PlexA2. In *Sema6A* and *PlexA2* null mutants, neurites from both Off and On SACs often fail to stratify into their appropriate sublaminae and exhibit "crossovers" as they extend between S2 and S4, normally the exclusive targets of Off SACs and On SACS, respectively (Sun et al. 2013) (Fig. 2.2).

In addition to regulating the sublaminar targeting of IPL amacrine cells and RGC neurites, Sema6A also controls the arborization of horizontal cell neurites in the OPL and On, but not Off, SACs in the IPL. Horizontal cells express both Sema6A and PlexA4, and in Sema6A and PlexA4 mutants horizontal cell neurites exhibit a reduction in self-avoidance, leading to an increase in dendrite self-crossing and also increased fasciculation among horizontal cell neurites; this results in an overall reduction in horizontal cell neurite coverage in the OPL (Matsuoka et al. 2012). A more dramatic dendritic arborization phenotype is observed in On SAC neurites of *Sema6A* and *PlexA2* mutants (Fig. 2.2). In these mutant On SACs, dendritic fields of individual SACs are dramatically reduced, accompanied by a loss of SAC dendritic arbor symmetry, a phenotype that suggests reduced dendrite self-avoidance during the course of On SAC dendritic elaboration during postnatal retinal development is regulated by Sema6A–PlexA2 signaling (Sun et al. 2013). Interestingly, On–Off direction-selective (DS) RGCs in *Sema6A* mutants exhibit normal Off DS tuning responses, however, On direction selective tuning responses are compromised (Fig. 2.2). Therefore, the On SAC morphological defects observed in *Sema6A* and *PlexA2* mutants correlate with defective Sema6A On DS responses, demonstrating how semaphorin–plexin signaling is critical for establishing distinct On and Off DS responses by On–Off DS RGCs. These On SAC dendritic arborization phenotypes appear quite distinct from those observed in mice harboring mutations that affect protocadherin expression (Lefebvre et al. 2012), suggesting that distinct repulsive signaling mechanisms together contribute to the

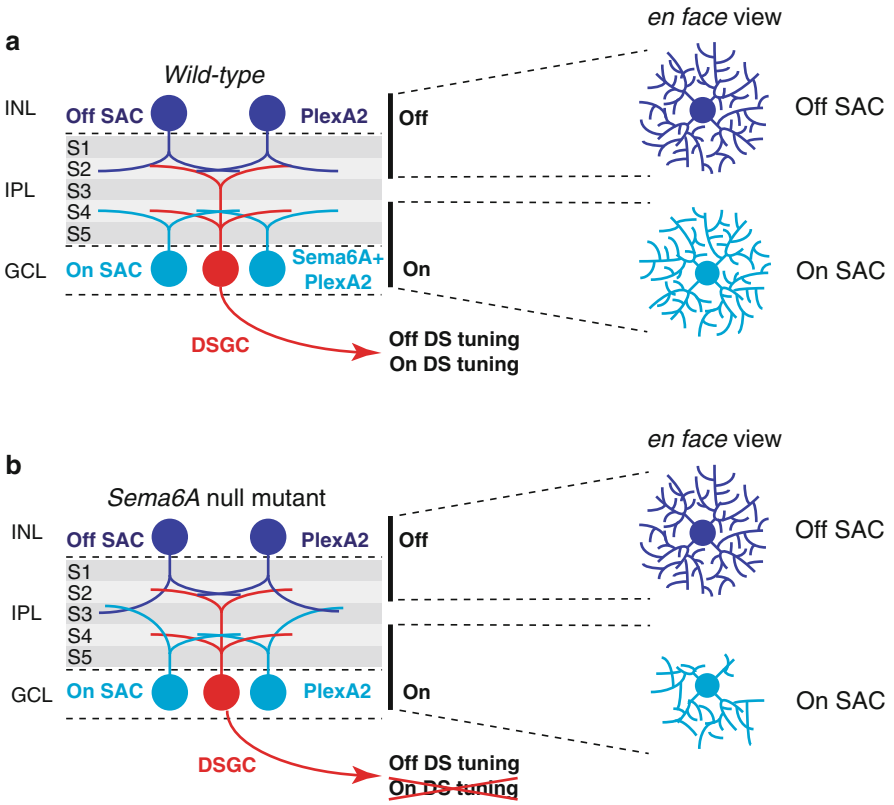

Fig. 2.2 Sema6A ensures On–Off SAC stratification and On SAC dendrite self-avoidance. (**a**) Schematic diagram shows sublaminar targeting in wild-type mouse retinas of Off SACs in S2, of On SACs in S4, and of direction-selective retinal ganglion cells (*DSGC*) in both S2 and S4. An en face view of Off SACs and On SACs reveals dendrite elaboration within their respective sublaminae. (**b**) In *Sema6A* null mutants, Off and On SACs fail to stratify, and On SACs neurites display self-avoidance defects and hyperfasciculation of dendritic processes. These defects ultimately lead to defects in DSGC On, but not Off, direction-selective (*DS*) tuning responses by On-Off DSGCs

radially symmetrical nature of nonoverlapping SAC dendritic morphology. These results, taken together, demonstrate the importance of transmembrane semaphorins in critical short-range repulsive guidance events that contribute to the establishment of proper laminar stratification, neuronal morphology, and connectivity within the retina.

Semaphorins and their receptors also are important in the guidance of central RGC projections. As RGC axons leave the eye and project centrally, they must navigate through the optic chiasm, a site where the majority of RGC axons cross the central nervous system (CNS) midline. The transmembrane semaphorin Sema6D, its PlexA1 receptor, and the NrCAM Ig superfamily adhesion molecule are key

mediators of midline crossing by RGC axons (Kuwajima et al. 2012). Sema6D is expressed by glial cells located at the CNS midline within the optic chiasm. PlexA1, a known Sema6D receptor (Toyofuku et al. 2004), and NrCAM, which binds Sema6D (Kuwajima et al. 2012), are both expressed in RGC axons. In either *Sema6D* mutants or *PlexA1*; *NrCAM* double mutants, RGC axons that normally cross the midline at the optic chiasm and project to central targets were found to either defasciculate at the optic chiasm or to fail to cross the midline altogether and instead project toward ipsilateral targets (Kuwajima et al. 2012). These phenotypes result from a loss not only of Sema6A-mediated midline repulsion, but also additional Sema6A attractive effects on RGC axons that are dependent upon PlexA1 and NrCAM, both of which are also expressed in the chiasm. In the zebrafish, secreted semaphorins also regulate RGC axon CNS midline crossing. Sema3D and Sema3E are expressed in tissue surrounding the optic chiasm. In zebrafish where either Sema3D or Sema3E is knocked down, or in *Sema3D* null mutants, the percentage of ipsilaterally projecting RGC axons is dramatically increased (Sakai and Halloran 2006; Dell et al. 2013). Neuropilin-1a (Nrp1a), a receptor for Sema3D and Sema3E, is expressed in zebrafish RCGs, and knockdown of Nrp1a results in an increase in the number of ipsilaterally projecting RGC axons (Dell et al. 2013). Interestingly, in the mouse visual system Nrp1 is required for RGC axons to successfully navigate through the optic chiasm; however, this is apparently achieved through the action of VEGF164, not class three secreted semaphorins (Erskine et al. 2011). These findings further demonstrate the importance of both transmembrane and secreted semaphorins for the guidance of RGC axons as they navigate toward their central nervous system targets.

2.3 Semaphorins Direct Wiring of the Developing Olfactory System

The secreted semaphorins Sema3A and Sema3F and their receptors Nrp1 and Nrp2, respectively, serve well-established functions during the formation of neural circuits within the mouse olfactory system (Schwarting et al. 2000, 2004; Cloutier et al. 2002, 2004; Walz et al. 2002, 2007; Matsuo et al. 2012). These studies demonstrate the importance of secreted semaphorins in the guidance and selective fasciculation of olfactory receptor neuron (ORN) axons that target both the main olfactory bulb (MOB) and the accessory olfactory bulb (AOB) of mice. Here, we explore recent findings showing that both secreted and transmembrane semaphorins regulate olfactory system innervation through axon–axon interactions and dendritic targeting.

The olfactory systems of vertebrates and invertebrates share a common neural circuit blueprint (Imai et al. 2010). Each ORN is located in a sensory organ and expresses a single functional odorant receptor (OR). ORNs project an axon to specialized central targets called glomeruli, regions where ORN axons connect to

dendrites of projection neurons. Interestingly, multiple ORNs expressing the same OR innervate the same specific glomerulus, and this is conserved from animal to animal. Together, all these observations lead to the principle that each ORN expresses a single OR, and OR expression defines innervation of specific individual glomeruli during olfactory system wiring. Although the mouse olfactory system, with more than 2 million ORNs, 1,000 ORs, and 2,000 glomeruli, is larger in scale than that of *Drosophila*, which contains 1,300 ORNs, 62 ORs, and 50 glomeruli, both have evolved somewhat similar strategies for ORN axon segregation to ensure the fidelity of ORN axon targeting to specific glomeruli.

Several studies from the Sakano laboratory have elucidated essential functions for secreted semaphorins in regulating the segregation of ORN axons as they project from the mouse olfactory epithelium to their targets in the MOB. With respect to ORN projections to the MOB along the anterior-posterior axis, graded levels of ORN intracellular cAMP, low anterior to high posterior with respect to MOB targeting, are generated from non-ligand-induced OR activity; this results in defined gradients of Nrp1 and Sema3a expression in ORNs (Imai et al. 2006). ORs are G protein-coupled receptors (GPCRs) that utilize the α-G-protein subunit G_{olf} to induce neural signaling in postnatal animals; however, during embryogenesis it is the α-G-protein subunit G_s that is critical for the generation of cAMP through basal, agonist-independent, activity of ORs (Nakashima et al. 2013). ORNs with high levels of cAMP, which induce high levels of Nrp1 expression, innervate glomeruli in more posterior regions of the dorsal MOB. Interestingly, ORNs with low levels of cAMP, which results in high Sema3a expression, innervate glomeruli in more anterior regions of the MOB (Imai et al. 2006, 2009). Mutant ORNs lacking Nrp1 or G_s have an altered trajectory and innervate glomeruli anterior to their normal target glomerulus. A striking phenotype observed in mouse mutants where Nrp1 or Sema3A is removed only in ORNs occurs within the ORN axon bundle itself, and this can be observed as ORN axons project toward the MOB. Within the ORN axon bundle, the Nrp1-expressing axons, which are destined to innervate posterior MOB glomeruli, are fully segregated from Sema3A-expressing ORN axons, which will innervate the anterior glomeruli. This segregation is dependent on Sema3A expression in ORNs, and it occurs before ORN axons reach the MOB (Fig. 2.3). Further, this segregation still occurs in mice that lack their MOB (Imai et al. 2009). Taken together, these results suggest that axon–axon interactions mediated by Sema3A and Nrp1 are critical for correct innervation of MOB glomeruli by ORNs. In addition to Sema3A, the secreted semaphorin Sema3F and its holoreceptor complex Nrp2/PlexA3 also play an essential, but distinct, role in topographic innervation of the MOB, here along the dorsal-ventral axis. Sema3F expression is limited to a subset of ORN axons that first arrive in the dorsal MOB; later-arriving ORNs do not express Sema3F at high levels but do express high levels of Nrp2 (Fig. 2.3). In *Sema3F*, *Nrp2*, and *PlexA3* mutants there is a dorsal shift in glomeruli innervation. These results implicate Sema3F release from ORNs as being required for topographic ORN innervation along the dorsal-ventral axis in the MOB. Taken together, these results demonstrate the importance of secreted semaphorins

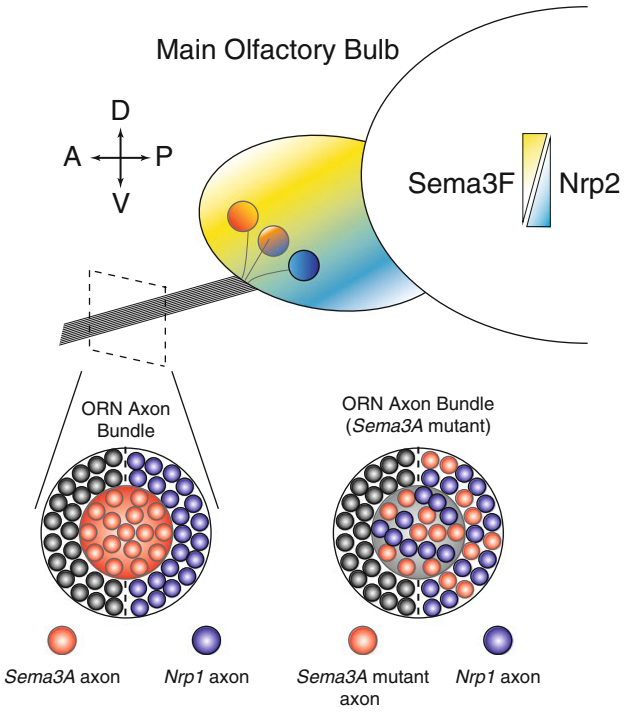

Fig. 2.3 Sema3A and Sema3F regulate olfactory neuron axon glomerular innervation in the mouse main olfactory bulb. Olfactory receptor neuron (ORN) axons fully segregate as they project toward the main olfactory bulb (MOB) in a Sema3A/Nrp1-dependent manner. This pre-target sorting of ORN axons results in axons that express high levels of Sema3A-innervating glomeruli in the anterior MOB and axons that express high levels of Nrp1-innervating posterior MOB glomeruli. ORN axons that innervate dorsal MOB glomeruli arrive in the MOB first and express high levels of Sema3F. ORN axons expressing high levels of Nrp2 arrive later and innervate the ventral glomeruli, a targeting event that requires ORN-expressed Sema3F

in regulating topographic innervation of the mouse MOB through ORN axon–axon interaction mechanisms.

Semaphorins also are critical for establishing neural connectivity in the invertebrate olfactory system. Interestingly, semaphorin regulation of axon–axon interactions is essential for regulating topographic innervation of glomeruli in the antennal lobe by ORNs residing in the third antennal segment and also in the maxillary palp of the developing *Drosophila* olfactory system. The transmembrane semaphorin Sema-1a and its PlexA receptor are required for proper topographic innervation of the antennal lobe by maxillary palp ORNs. Using a molecular mechanism that is reminiscent of Sema3F regulation of ORN axon targeting in the mouse MOB (see above), Sema-1a expression on axons from ORNs in the third antenna segment is essential for proper targeting of glomeruli by axons from ORNs in the maxillary palp (Fig. 2.4a). Sema-1a-expressing ORN axons from the third segment of the

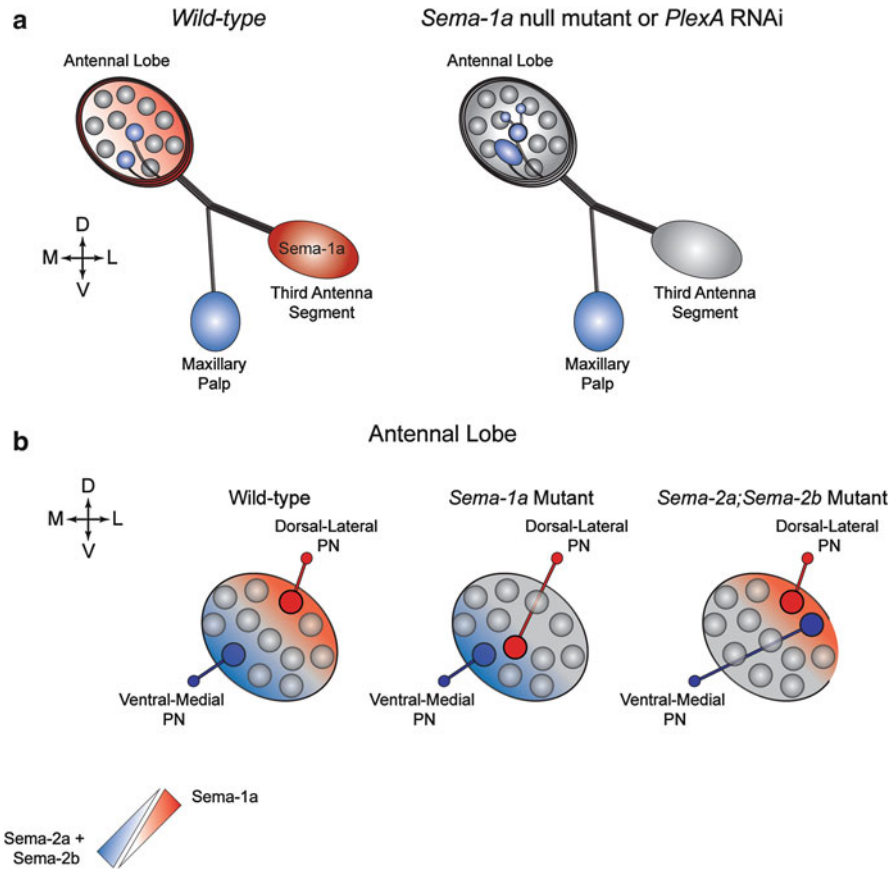

Fig. 2.4 Semaphorins regulate glomerular targeting by olfactory receptor neuron axons and projection neuron dendrites in the *Drosophila* antennal lobe. (**a**) In wild-type animals, ORN axons from the third antennal segment express Sema-1a and innervate the antennal lobe before the arrival of ORN axons from the maxillary palp. Sema-1a-expressing axons from the third antennal segment serve to constrain maxillary palp ORN axons, allowing them to project to their appropriate glomeruli. In *Sema-1a* null mutant pupae, or pupae expressing PlexA RNAi, ORN axons from the maxillary palp fail to innervate their appropriate target glomeruli. (**b**) Projection neurons (PNs) selectively target their dendrites to appropriate antennal lobe locations. Within the antennal lobe there is a high dorsolateral to low ventromedial gradient of Sema-1a expression and a high ventromedial to low dorsolateral Sema2a–Sema2b gradient. In *Sema-1a* null mutant animals, PNs that typically target the dorsolateral antennal lobe innervate ventromedial regions. Further, in *Sema2a*; *Sema2b* double-null mutants, PNs that normally target ventromedial antennal lobe regions instead innervate more dorsolateral target areas

antenna are the first to arrive in the antennal lobe, and they serve to constrain later-arriving ORN axons from the maxillary palp through Sema-1a-mediated axon–axon interactions within the antennal lobe (Lattemann et al. 2007; Sweeney et al. 2007). The secreted semaphorin Sema2b and its receptor PlexB also regulate axon–axon

interactions during ORN innervation of the antennal lobe. ORN axons extending from the antenna take either a dorsolateral or ventromedial trajectory to innervate the antennal lobe. Only those ORN axons that take the ventromedial trajectory express Sema2b. Sema2b and PlexB expression in antenna ORN axons are both required for proper trajectory and glomerular targeting by axons in the ventromedial region of the antennal lobe; they are dispensable for innervation of the dorsolateral region of the antennal lobe. Sema2b functions as an attractive cue, and in addition to being expressed on ventromedial ORN axons it is also expressed in the ventromedial target region of the antennal lobe. Therefore, Sema2b mediates both ORN axon–axon interactions and ORN axon–target interactions, demonstrating how a single guidance cue can function at multiple stages of axon targeting to drive select neural circuit assembly (Joo et al. 2013). These results in both *Drosophila* and mouse olfactory systems demonstrate highly conserved molecular mechanisms by which semaphorins regulate topographic mapping through a series of axon–axon interactions, both along ORN trajectories and between ORNs and their targets.

Recent work also illuminates a role for semaphorins in regulating dendritic targeting in the *Drosophila* antennal lobe. Projection neurons (PNs) are located proximal to the antennal lobe, target their dendrites to individual olfactory glomeruli, and have an axon that targets both the mushroom body and the lateral horn. PN dendrites target individual glomeruli before they are innervated by pupal ORNs. Both secreted and transmembrane semaphorins establish overlapping gradients in the developing antennal lobe that regulate PN dendritic targeting (Komiyama et al. 2007; Sweeney et al. 2011). Sema-1a is expressed in a high dorsolateral to low ventromedial gradient across the antennal lobe in PN dendrites. In the absence of Sema-1a, PN dendrites that would normally target the dorsolateral antennal lobe instead innervate more ventral regions (Fig. 2.4b). Expression of full-length Sema-1a in PN neurons was sufficient to rescue this defect, but rescue experiments using Sema-1a lacking its intracellular domain failed to rescue the dendritic targeting phenotype (Komiyama et al. 2007). This work provided the initial example of transmembrane semaphorin reverse signaling. In the antennal lobe, the secreted semaphorins Sema2a and Sema2b are expressed in a high ventromedial to low dorsolateral gradient, complementary to the Sema-1a expression pattern. In *Sema2a*; *Sema2b* double-null mutants, dendrites from PNs that normally target the ventromedial regions of the antennal lobe innervate dorsal regions, suggesting that Sema2a and Sema2b serve to constrain targeting of ventromedial projecting PN dendrites away from the dorsal antennal lobe (Sweeney et al. 2011) (Fig. 2.4b). Although Sema2a expression in larval ORN axons was sufficient to rescue the Sema2a–Sema2b double-mutant PN dendritic targeting phenotype, a striking result from this study was that the known semaphorin receptors PlexA and PlexB were dispensable for ventromedial PN dendrite targeting. Interestingly, the *Sema2a*; *Sema2b* double-mutant PN dendritic targeting phenotype is qualitatively similar to that observed following loss of Sema-1a in PNs, Sema2a/2b expression is opposite to that of Sema-1a in the developing antennal lobe, and the extracellular domain of Sema-1a binds to Sema2a exogenously expressed by cells in fly wing disc and in brain neurons that overexpress Sema2a. This observation suggests the intriguing

possibility that Sema2a and Sema2b signal through Sema-1a to control dendritic targeting, and future work will confirm the identity of the Sema2a/2b receptor or receptor complex that serves to guide PN dendrites to their appropriate antennal lobe regions. Taken together, these studies show an important role for both secreted and transmembrane semaphorins in directing topographic dendritic targeting in the developing *Drosophila* olfactory system.

2.4 Semaphorin Regulation of Neural Circuit Formation in the Brain

Semaphorins direct the formation of a wide range of neural circuits within the mammalian brain. Semaphorin signaling is required for many conserved aspects of neural circuit assembly, including the successful guidance of axons to their innervation sites, formation of synapses, and the refinement of neural circuits through pruning of axons and synapses. In recent years, many studies have identified roles for semaphorins in these processes, and novel molecular mechanisms of semaphorin signaling have been uncovered. Here, we consider some of these findings that provide new insights into the molecular mechanisms utilized by semaphorins during neural circuit formation in the mammalian brain.

To regulate axon guidance, class three secreted semaphorins typically signal through a holoreceptor complex by binding directly to either Nrp1 or Nrp2 and forming a complex with a PlexA receptor family member that mediates signal transduction (Tran et al. 2007). However, there are exceptions to this rule, and there is growing evidence that interactions between semaphorin receptors and additional transmembrane receptors modulate axon guidance responses to class three semaphorins. One notable exception is the secreted semaphorin Sema3E, which can bind directly to the PlexD1 receptor independent of Nrp1 or Nrp2 (Gu et al. 2005). Although Sema3E and PlexD1 were originally characterized for their roles in regulating vascular development, they are now known to play critical roles in the development of several neural circuits in the brain. In a recent study, Sema3E expression in the striatum was shown to be required for the convergence of cortical thalamic axons (CTAs) and thalamocortical axons (TCAs); this is achieved by stalling PlexD1-expressing CTAs in the striatum until TCAs arrive to converge and guide the CTAs along their appropriate trajectory (Deck et al. 2013). Similar to many other axon guidance cues, Sema3E can serve as either an attractive or repulsive guidance cue for axons navigating through the brain. For example, Sema3E was shown to mediate attraction through a novel signaling complex. In the brain, the cortical fugal and subiculomammillary tracts run in close proximity to one another, and they both express PlexD1. The cortical neurons are repelled by Sema3E; however, the subicular neurons are attracted to Sema3E (Chauvet et al. 2007). How is this differential response achieved? PlexD1 is required for both responses, and PlexD1 mutants have defects in both the cortical fugal and

subiculomammillary tracts. Interestingly, subicular neurons also express Nrp1, and the application of exogenous Nrp1 ectodomain is sufficient to convert Sema3E from a repellent to an attractant for cortical neurons (Chauvet et al. 2007; Bellon et al. 2010). The vascular endothelial growth factor (VEGF) receptor 2 (VEGFR2) is also necessary for Sema3E-mediated attraction of subicular neurons, and Sema3E can form a complex with PlexD1/Nrp1/VEGFR2 in a heterologous system (Bellon et al. 2010). These studies demonstrate the importance of Sema3E as a bifunctional guidance cue for establishing neural circuits within the brain, and they provide evidence of unconventional semaphorin signaling.

The secreted semaphorin Sema3F was also shown to have a bifunctional role in regulating innervation in the dopaminergic system. Mesodiencephalic dopaminergic (mdDA) neurons located in the medial ventral tegmental area (mVTA) project axons into the prefrontal cortex (PFC). In either Sema3F or Nrp2 mutants, mdDA neuron axons display both fasciculation and targeting defects along the trajectory of this neural circuit (Kolk et al. 2009). An interesting result in this study is an apparent change in response to Sema3F by mdDA neurons in the mVTA. At early embryonic time points Sema3F is attractive to mdDA neurons, but as the embryos developed this attractive response to Sema3F shifts to repulsion. The precise molecular mechanism that mediates this transition from attraction to repulsion remains to be elucidated.

In the spinal cord and in the brain, L1 Ig superfamily adhesion molecule family members have been shown to be modulators of class three semaphorin signaling (Castellani et al. 2000; Falk et al. 2005). Since these studies were presented, additional evidence supporting the importance of Ig superfamily-mediated adhesion in neuronal guidance has been obtained in the context of TCA topographic projections into the cortex. The *Close homologue of L1 (CHL1)* gene is expressed in thalamic neurons along with Nrp1. CHL1 was found to form a complex with Nrp1 and mediate Sema3A growth cone collapse in cultured thalamic neurons. In either *CHL1* mutants or $Nrp1^{Sema}$ mutants (an *Nrp1* allele deficient in secreted semaphorin binding) (Gu et al. 2003), somatosensory TCAs become misrouted and innervate the visual cortex (Wright et al. 2007). In a related study, another L1 adhesion family member, NrCAM, was found to be coexpressed with Nrp2 in thalamic neurons and to form an NrCAM/Nrp2 protein complex. NrCAM was also found to be necessary for Sema3F-mediated growth cone collapse of cultured thalamic neurons. In vivo, motor and somatosensory TCAs from both NrCAM and Nrp2 mutants aberrantly innervate the visual cortex (Demyanenko et al. 2011). These studies provide further evidence that L1 adhesion molecule family members are important modulators of semaphorin-regulated axon guidance.

In addition to sculpting the overall trajectories that define a wide range of axon tracts in the mammalian brain, semaphorins also play instrumental roles in controlling the precise innervation of laminated structures in the brain. In the cerebellum, basket cells located in the molecular layer densely innervate the axon hillock of Purkinje cells located in the Purkinje cell layer (PCL). Basket cells express Nrp1, but Sema3A is expressed specifically in the PCL. In either Sema3A or $Nrp1^{Sema}$ mutants, a dramatic reduction in basket cell axon branching occurs

in the PCL, resulting in a reduction in innervation of the Purkinje cell axon hillock (Cioni et al. 2013). In contrast to class three secreted semaphorin promotion of laminar innervation in the cerebellum, transmembrane semaphorins serve to constrain laminar innervation in the hippocampus. In the hippocampus, axons from dentate gyrus (DG) granule cell (GC) neurons project within the two mossy fiber tracts into the CA3 region. The main bundle, or suprapyramidal tract, innervates the apical dendrites of CA3 pyramidal neurons, whereas the much less organized infrapyramidal tract (IPT) innervates the basal dendrites of CA3 pyramidal neurons. The transmembrane semaphorins Sema6A and Sema6B are expressed in CA3 pyramidal neurons, and they serve to constrain the main mossy fiber tract to the surprapyramidal region (Suto et al. 2007; Tawarayama et al. 2010). Interestingly, both *PlexA2* and *PlexA4* single mutants exhibit a more severe phenotype than is observed in the *Sema6A*; *Sema6B* double-null mutant, suggesting that additional ligands may serve to regulate mossy fiber laminar innervation in CA3. Future studies will determine whether other semaphorins also contribute to hippocampal laminar innervation.

Semaphorins also regulate the precise innervation of target tissues through the refinement of synapses following initial innervation events. In the mouse hippocampus, a signaling cascade that regulates pruning of the infrapyarmidal tract (IPT) was recently elucidated. During postnatal development, DG axons in the IPT extend along the basal dendrites of CA3 pyramidal neurons. However, as the mice approach adulthood (between postnatal days 20 and 40), the IPT undergoes retraction-mediated axon pruning and is significantly shortened (Bagri et al. 2003). This response is dependent on Sema3F signaling through the Nrp2/PlexA3 holoreceptor complex (Bagri et al. 2003; Liu et al. 2005; Riccomagno et al. 2012). β2-Chimaerin (β2Chn) is a Rho GTPase-activating protein (GAP) specific for Rac, and Sema3F robustly activates its GTPase activity. β2Chn is required for Sema3F/Nrp2/PlexA3 IPT pruning, and this IPT pruning is achieved through the downregulation of Rac activity (Riccomagno et al. 2012). Remarkably, β2Chn is dispensable for Sema3F-mediated repulsive axon guidance, and also for Sema3F-mediated dendritic spine constraint, and therefore these observations provide the first example of a semaphorin-mediated signaling mechanism that selectively regulates axon pruning. In addition to retraction-mediated pruning, Nrp2 and PlexA3 are necessary for degeneration-like pruning of mouse cortical neurons. Layer V pyramidal neurons in the visual cortex initially innervate regions of both the motor and visual systems through the formation of extensive collateral axon branches. During postnatal development, collateral axons from visual cortex layer V pyramidal neurons that innervate the motor system undergo Nrp2/PlexA3-dependent degeneration-like pruning (Low et al. 2008). Nrp2 and PlexA3 mutants fail to prune axons extending from the visual cortex into areas devoted to motor systems. It remains to be determined exactly which semaphorin signaling events govern these cortical axon pruning events. These examples demonstrate the importance of semaphorin signaling in the refinement of neural circuit formation in the brain.

In addition to regulating axon guidance and pruning in the CNS, semaphorins also modulate neural circuit formation by selectively regulating synapse formation

in several systems within the brain. For example, Sema3F signaling through its Nrp2/PlexA3 holoreceptor complex constrains dendritic spine elaboration and synaptogenesis in layer V pyramidal neurons of the cerebral cortex and hippocampal DG cells (Tran et al. 2009). In *Sema3F*, *Nrp2*, and *PlexA3* mutants, a significant increase in the density of spines and glutamatergic excitatory synapses is observed. Strikingly, in layer V pyramidal neurons the increase in dendritic spine density is observed only along the apical dendrite in vivo, and in cultured deep layer cortical neurons the Nrp2 co-receptor is also observed to be localized only on apical dendrites and not on basal dendritic processes (Tran et al. 2009). Therefore, Sema3F signaling through Nrp2/PlexA3 selectively constrains excitatory synapses and dendritic spines to a subdomain within cortical pyramidal neuron dendrites. Sema3E and PlexD1 also selectively limit excitatory synapse formation. Within the adult striatum, PlexD1 is exclusively expressed within the medium spiny neurons (MSNs) in the direct dopaminergic pathway. These MSNs receive excitatory glutamatergic input from both cortical neurons and thalamic neurons. In *Sema3E* and *PlexD1* mutants, MSNs in the direct pathway exhibit an increase in excitatory synaptic input from thalamic neurons, and MSNs display an increase in synapses formed selectively on their somas and dendritic shafts (Ding et al. 2012). The thalamic axons are a source of Sema3E in the adult striatum, and Sema3E/PlexD1 signaling apparently plays an important feedback role in balancing excitatory synaptic input in direct pathway MSNs. Although class three semaphorins are important for constraining excitatory input, there is now growing evidence that the transmembrane semaphorin Sema4D promotes inhibitory synapse formation. In cultured hippocampal neurons, siRNA-mediated knockdown of Sema4D reduces GABAergic inhibitory synaptic formation (Paradis et al. 2007). Remarkably, the application of exogenous Sema4D rapidly induces the formation of inhibitory synapses in a matter of hours. This effect is completely abolished in cultured neurons or hippocampal sections prepared from mice lacking the Sema4D receptor PlexB1 (Kuzirian et al. 2013). These studies, taken together, demonstrate that semaphorins are required for the selective establishment of excitatory and inhibitory synapses in the mammalian brain.

2.5 Conclusion

More than 20 years have passed since semaphorins were first identified as regulators of axon growth cone guidance (Kolodkin et al. 1992). However, new neuronal functions for these proteins are still being identified. We have highlighted recent and novel findings regarding semaphorin function in the central nervous system. Semaphorins play critical roles in all aspects of neural circuit assembly, including axon targeting, laminar-specific innervation, selective synapse formation, and refinement of neural circuits through axon and synapse pruning. We expect that new roles for semaphorins in neural circuit establishment, maintenance, and plasticity

remain to be discovered, and it seems likely that novel semaphorin signaling mechanisms will be elucidated that underlie these functions.

Recent work demonstrates that Sema6A also plays a critical role in assembly of accessory optic system RGC circuits (Sun et al. 2015). Sema6A is expressed in On direction selective retinal ganglion cells and serves as a receptor in these neurons to establish connectivity with their brainstem target, the medial terminal nucleus.

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References

Bagri A, Cheng HJ, Yaron A, Pleasure SJ, Tessier-Lavigne M (2003) Stereotyped pruning of long hippocampal axon branches triggered by retraction inducers of the semaphorin family. Cell 113(3):285–299

Bellon A, Luchino J, Haigh K, Rougon G, Haigh J, Chauvet S, Mann F (2010) VEGFR2 (KDR/Flk1) signaling mediates axon growth in response to semaphorin 3E in the developing brain. Neuron 66(2):205–219

Cafferty P, Yu L, Long H, Rao Y (2006) Semaphorin-1a functions as a guidance receptor in the Drosophila visual system. J Neurosci 26(15):3999–4003

Castellani V, Chedotal A, Schachner M, Faivre-Sarrailh C, Rougon G (2000) Analysis of the L1-deficient mouse phenotype reveals cross-talk between Sema3A and L1 signaling pathways in axonal guidance. Neuron 27(2):237–249

Chauvet S, Cohen S, Yoshida Y, Fekrane L, Livet J, Gayet O, Segu L, Buhot MC, Jessell TM, Henderson CE, Mann F (2007) Gating of Sema3E/PlexinD1 signaling by neuropilin-1 switches axonal repulsion to attraction during brain development. Neuron 56(5):807–822

Cioni JM, Telley L, Saywell V, Cadilhac C, Jourdan C, Huber AB, Huang JZ, Jahannault-Talignani C, Ango F (2013) SEMA3A signaling controls layer-specific interneuron branching in the cerebellum. Curr Biol 23(10):850–861

Cloutier JF, Giger RJ, Koentges G, Dulac C, Kolodkin AL, Ginty DD (2002) Neuropilin-2 mediates axonal fasciculation, zonal segregation, but not axonal convergence, of primary accessory olfactory neurons. Neuron 33(6):877–892

Cloutier JF, Sahay A, Chang EC, Tessier-Lavigne M, Dulac C, Kolodkin AL, Ginty DD (2004) Differential requirements for semaphorin 3F and Slit-1 in axonal targeting, fasciculation, and segregation of olfactory sensory neuron projections. J Neurosci 24(41):9087–9096

Deck M, Lokmane L, Chauvet S, Mailhes C, Keita M, Niquille M, Yoshida M, Yoshida Y, Lebrand C, Mann F, Grove EA, Garel S (2013) Pathfinding of corticothalamic axons relies on a rendezvous with thalamic projections. Neuron 77(3):472–484

Dell AL, Fried-Cassorla E, Xu H, Raper JA (2013) cAMP-induced expression of neuropilin1 promotes retinal axon crossing in the zebrafish optic chiasm. J Neurosci 33(27):11076–11088

Demyanenko GP, Riday TT, Tran TS, Dalal J, Darnell EP, Brennaman LH, Sakurai T, Grumet M, Philpot BD, Maness PF (2011) NrCAM deletion causes topographic mistargeting of thalamo-cortical axons to the visual cortex and disrupts visual acuity. J Neurosci 31(4):1545–1558

Ding JB, Oh WJ, Sabatini BL, Gu C (2012) Semaphorin 3E-Plexin-D1 signaling controls pathway-specific synapse formation in the striatum. Nat Neurosci 15(2):215–223

Erskine L, Reijntjes S, Pratt T, Denti L, Schwarz Q, Vieira JM, Alakakone B, Shewan D, Ruhrberg C (2011) VEGF signaling through neuropilin 1 guides commissural axon crossing at the optic chiasm. Neuron 70(5):951–965

Falk J, Bechara A, Fiore R, Nawabi H, Zhou H, Hoyo-Becerra C, Bozon M, Rougon G, Grumet M, Puschel AW, Sanes JR, Castellani V (2005) Dual functional activity of semaphorin 3B is required for positioning the anterior commissure. Neuron 48(1):63–75

Gu C, Rodriguez ER, Reimert DV, Shu T, Fritzsch B, Richards LJ, Kolodkin AL, Ginty DD (2003) Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. Dev Cell 5(1):45–57

Gu C, Yoshida Y, Livet J, Reimert DV, Mann F, Merte J, Henderson CE, Jessell TM, Kolodkin AL, Ginty DD (2005) Semaphorin 3E and plexin-D1 control vascular pattern independently of neuropilins. Science 307(5707):265–268

Imai T, Suzuki M, Sakano H (2006) Odorant receptor-derived cAMP signals direct axonal targeting. Science 314(5799):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(5940):585–590

Imai T, Sakano H, Vosshall LB (2010) Topographic mapping – the olfactory system. Cold Spring Harbor Perspect Biol 2(8):a001776

Jeong S, Juhaszova K, Kolodkin AL (2012) The control of semaphorin-1a-mediated reverse signaling by opposing pebble and RhoGAPp190 functions in drosophila. Neuron 76(4):721–734

Joo WJ, Sweeney LB, Liang L, Luo L (2013) Linking cell fate, trajectory choice, and target selection: genetic analysis of Sema-2b in olfactory axon targeting. Neuron 78(4):673–686

Kolk SM, Gunput RA, Tran TS, van den Heuvel DM, Prasad AA, Hellemons AJ, Adolfs Y, Ginty DD, Kolodkin AL, Burbach JP, Smidt MP, Pasterkamp RJ (2009) Semaphorin 3F is a bifunctional guidance cue for dopaminergic axons and controls their fasciculation, channeling, rostral growth, and intracortical targeting. J Neurosci 29(40):12542–12557

Kolodkin AL, Matthes DJ, O'Connor TP, Patel NH, Admon A, Bentley D, Goodman CS (1992) Fasciclin IV: sequence, expression, and function during growth cone guidance in the grasshopper embryo. Neuron 9(5):831–845

Komiyama T, Sweeney LB, Schuldiner O, Garcia KC, Luo L (2007) Graded expression of semaphorin-1a cell-autonomously directs dendritic targeting of olfactory projection neurons. Cell 128(2):399–410

Kuwajima T, Yoshida Y, Takegahara N, Petros TJ, Kumanogoh A, Jessell TM, Sakurai T, Mason C (2012) Optic chiasm presentation of Semaphorin6D in the context of Plexin-A1 and Nr-CAM promotes retinal axon midline crossing. Neuron 74(4):676–690

Kuzirian MS, Moore AR, Staudenmaier EK, Friedel RH, Paradis S (2013) The class 4 semaphorin Sema4D promotes the rapid assembly of GABAergic synapses in rodent hippocampus. J Neurosci 33(21):8961–8973

Lattemann M, Zierau A, Schulte C, Seidl S, Kuhlmann B, Hummel T (2007) Semaphorin-1a controls receptor neuron-specific axonal convergence in the primary olfactory center of Drosophila. Neuron 53(2):169–184

Lefebvre JL, Kostadinov D, Chen WV, Maniatis T, Sanes JR (2012) Protocadherins mediate dendritic self-avoidance in the mammalian nervous system. Nature 488(7412):517–521

Liu XB, Low LK, Jones EG, Cheng HJ (2005) Stereotyped axon pruning via plexin signaling is associated with synaptic complex elimination in the hippocampus. J Neurosci 25(40):9124–9134

Low LK, Liu XB, Faulkner RL, Coble J, Cheng HJ (2008) Plexin signaling selectively regulates the stereotyped pruning of corticospinal axons from visual cortex. Proc Natl Acad Sci U S A 105(23):8136–8141

Matsuo T, Rossier DA, Kan C, Rodriguez I (2012) The wiring of Grueneberg ganglion axons is dependent on neuropilin 1. Development 139(15):2783–2791

Matsuoka RL, Chivatakarn O, Badea TC, Samuels IS, Cahill H, Katayama K, Kumar SR, Suto F, Chedotal A, Peachey NS, Nathans J, Yoshida Y, Giger RJ, Kolodkin AL (2011a) Class 5 transmembrane semaphorins control selective Mammalian retinal lamination and function. Neuron 71(3):460–473

Matsuoka RL, Nguyen-Ba-Charvet KT, Parray A, Badea TC, Chedotal A, Kolodkin AL (2011b) Transmembrane semaphorin signalling controls laminar stratification in the mammalian retina. Nature 470(7333):259–263

Matsuoka RL, Jiang Z, Samuels IS, Nguyen-Ba-Charvet KT, Sun LO, Peachey NS, Chedotal A, Yau KW, Kolodkin AL (2012) Guidance-cue control of horizontal cell morphology, lamination, and synapse formation in the mammalian outer retina. J Neurosci 32(20):6859–6868

Nakashima A, Takeuchi H, Imai T, Saito H, Kiyonari H, Abe T, Chen M, Weinstein LS, Yu CR, Storm DR, Nishizumi H, Sakano H (2013) Agonist-independent GPCR activity regulates anterior-posterior targeting of olfactory sensory neurons. Cell 154(6):1314–1325

Paradis S, Harrar DB, Lin Y, Koon AC, Hauser JL, Griffith EC, Zhu L, Brass LF, Chen C, Greenberg ME (2007) An RNAi-based approach identifies molecules required for glutamatergic and GABAergic synapse development. Neuron 53(2):217–232

Pecot MY, Tadros W, Nern A, Bader M, Chen Y, Zipursky SL (2013) Multiple interactions control synaptic layer specificity in the Drosophila visual system. Neuron 77(2):299–310

Riccomagno MM, Hurtado A, Wang H, Macopson JG, Griner EM, Betz A, Brose N, Kazanietz MG, Kolodkin AL (2012) The RacGAP beta2-Chimaerin selectively mediates axonal pruning in the hippocampus. Cell 149(7):1594–1606

Sakai JA, Halloran MC (2006) Semaphorin 3d guides laterality of retinal ganglion cell projections in zebrafish. Development 133(6):1035–1044

Sanes JR, Yamagata M (2009) Many paths to synaptic specificity. Annu Rev Cell Dev Biol 25:161–195

Sanes JR, Zipursky SL (2010) Design principles of insect and vertebrate visual systems. Neuron 66(1):15–36

Schwarting GA, Kostek C, Ahmad N, Dibble C, Pays L, Puschel AW (2000) Semaphorin 3A is required for guidance of olfactory axons in mice. J Neurosci 20(20):7691–7697

Schwarting GA, Raitcheva D, Crandall JE, Burkhardt C, Puschel AW (2004) Semaphorin 3A-mediated axon guidance regulates convergence and targeting of P2 odorant receptor axons. Eur J Neurosci 19(7):1800–1810

Sun LO, Brady CM, Cahill H, Al-Khindi T, Sakuta H, Dhande OS, Noda M, Huberman AD, Nathans J, Kolodkin AL (2015) Functional assembly of accessory optic system circuitry critical for compensatory eye movements. Neuron (in press)

Sun LO, Jiang Z, Rivlin-Etzion M, Hand R, Brady CM, Matsuoka RL, Yau KW, Feller MB, Kolodkin AL (2013) On and off retinal circuit assembly by divergent molecular mechanisms. Science 342(6158):1241974

Suto F, Tsuboi M, Kamiya H, Mizuno H, Kiyama Y, Komai S, Shimizu M, Sanbo M, Yagi T, Hiromi Y, Chedotal A, Mitchell KJ, Manabe T, Fujisawa H (2007) Interactions between plexin-A2, plexin-A4, and semaphorin 6A control lamina-restricted projection of hippocampal mossy fibers. Neuron 53(4):535–547

Sweeney LB, Couto A, Chou YH, Berdnik D, Dickson BJ, Luo L, Komiyama T (2007) Temporal target restriction of olfactory receptor neurons by Semaphorin-1a/PlexinA-mediated axon-axon interactions. Neuron 53(2):185–200

Sweeney LB, Chou YH, Wu Z, Joo W, Komiyama T, Potter CJ, Kolodkin AL, Garcia KC, Luo L (2011) Secreted semaphorins from degenerating larval ORN axons direct adult projection neuron dendrite targeting. Neuron 72(5):734–747

Tawarayama H, Yoshida Y, Suto F, Mitchell KJ, Fujisawa H (2010) Roles of semaphorin-6B and plexin-A2 in lamina-restricted projection of hippocampal mossy fibers. J Neurosci 30(20):7049–7060

Toyofuku T, Zhang H, Kumanogoh A, Takegahara N, Suto F, Kamei J, Aoki K, Yabuki M, Hori M, Fujisawa H, Kikutani H (2004) Dual roles of Sema6D in cardiac morphogenesis through region-specific association of its receptor, Plexin-A1, with off-track and vascular endothelial growth factor receptor type 2. Genes Dev 18(4):435–447

Tran TS, Kolodkin AL, Bharadwaj R (2007) Semaphorin regulation of cellular morphology. Annu Rev Cell Dev Biol 23:263–292

Tran TS, Rubio ME, Clem RL, Johnson D, Case L, Tessier-Lavigne M, Huganir RL, Ginty DD, Kolodkin AL (2009) Secreted semaphorins control spine distribution and morphogenesis in the postnatal CNS. Nature 462(7276):1065–1069

Walz A, Rodriguez I, Mombaerts P (2002) Aberrant sensory innervation of the olfactory bulb in neuropilin-2 mutant mice. J Neurosci 22(10):4025–4035

Walz A, Feinstein P, Khan M, Mombaerts P (2007) Axonal wiring of guanylate cyclase-D-expressing olfactory neurons is dependent on neuropilin 2 and semaphorin 3F. Development 134(22):4063–4072

Wright AG, Demyanenko GP, Powell A, Schachner M, Enriquez-Barreto L, Tran TS, Polleux F, Maness PF (2007) Close homolog of L1 and neuropilin 1 mediate guidance of thalamocortical axons at the ventral telencephalon. J Neurosci 27(50):13667–13679

Yu L, Zhou Y, Cheng S, Rao Y (2010) Plexin a-semaphorin-1a reverse signaling regulates photoreceptor axon guidance in Drosophila. J Neurosci 30(36):12151–12156