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Semaphorins and the Dynamic Regulation of Synapse Assembly, Refinement, and Function

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Abstract

Semaphorins are phylogenetically conserved proteins expressed in most organ systems, including the nervous system. Following their description as axon guidance cues, semaphorins have been implicated in multiple aspects of nervous system development. Semaphorins are key regulators of neural circuit assembly, neuronal morphogenesis, assembly of excitatory and inhibitory synapses, and synaptic refinement. Semaphorins contribute to the balance between excitatory and inhibitory synaptic transmission, and electrical activity can modulate semaphorin signaling in neurons. This interplay between guidance cue signaling and electrical activity has the potential to sculpt the wiring of neural circuits and to modulate their function.

Introduction

Neurons are polarized cells, facilitating the performance of specialized functions by different regions of the same cell. Unique combinations of dendritic and axonal morphologies regulate synaptic properties and coordinate neural transmission within circuits. Factors that affect complexity of dendritic arbors have a profound influence on the number and type of synaptic inputs a neuron receives [1]. Dendrites of many neurons exhibit small protuberances called spines; these are the sites of excitatory synaptic transmission. A growing number of molecules are now known to affect excitatory synapse formation in the mammalian central nervous system (CNS), including transmembrane proteins and also secreted proteins that can be derived from neurons or astrocytes [2,3,4]. Recent work shows that members of the semaphorin protein family, too, play critical roles in establishing neural connectivity and regulating synaptic development and function.

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Conflict of interest

The authors declare no competing financial interests (EK and ALK).

We consider here recent work showing how semaphorins regulate neuronal polarization, multiple aspects of dendritic morphology, excitatory and inhibitory synaptogenesis, and the establishment of precise connectivity among disparate neuronal classes. Following these initial stages of synapse formation, many axonal and dendritic circuit components are refined, or pruned, to generate mature circuits, and some of these events involve semaphorin signaling. Modulation of synaptic transmission provides neural circuits with the potential for experience-dependent plasticity, and recent work suggests that semaphorin signaling functions throughout life to sculpt circuits in response to experience. Together, all of these observations expand our view of the molecular landscape that influences neural circuit elaboration, uncovering novel influences on synapse development, function, and plasticity.

Semaphorins regulate neuronal polarity, dendritic arborization, and synapse distribution

The complexity of dendritic arbors greatly influences neuronal electrical properties. Ample evidence shows that semaphorin signaling affects the development of dendrites belonging to multiple and diverse neuronal classes. The Class 3 secreted semaphorin *Sema3A* promotes dendrite development with a concomitant inhibition of axon growth *in vitro* [5,6]. Further, *Sema3A* promotes the elaboration of cortical layer 5 pyramidal neuron basal dendrites during postnatal mouse development [7,8] and also dendritic development in newly born adult dentate gyrus (DG) granule cell (GC) [9]. Therefore, molecular programs that regulate dendritic development are likely conserved from embryogenesis to the adult.

In highly stratified regions of the nervous system, where synaptic specificity is achieved through neurite arborization of select neuronal populations in precisely defined layers, semaphorins function to constrain dendritic arbors within laminae. In the *Xenopus* retina, class 3 semaphorins regulate asymmetric growth of retinal ganglion cell (RGC) dendritic arbors [10]. In the mouse retina, the transmembrane semaphorins *Sema6A*, *Sema5A* and *Sema5B* direct precise neurite patterning of select retinal cell types [11,12,13]. In the *Drosophila* visual system, transmembrane *Sema-1a* guides growing L3 neuron axons to their laminar target in the M3 layer of the medulla [14•]. *Sema-1a* also acts in the *Drosophila* olfactory system to pattern the dendrites of olfactory projection neurons within the antennal lobe [15], responding to a gradient of the secreted semaphorins *Sema-2a* and *Sema-2b* in the dorsolateral antennal lobe [16•].

Semaphorins also influence synapse formation and subcellular synaptic localization. In *C. elegans* two transmembrane semaphorins, *Sema-1* and *Sema-2*, which signal through the plexin-1 receptor, act to restrict *en passant* synapse formation between complementary axonal regions of two adjacent motor neurons and the same muscle field [17••]. Further, in the mouse cerebral cortex the secreted semaphorin *Sema3F* regulates subcellular synapse specificity by constraining dendritic spine density selectively along apical dendrites of deep layer pyramidal neurons [8] (Figure 1a).

Pathway-specific connectivity: semaphorins and the CNS circuit development

During neural circuit assembly, growing axons recognize their postsynaptic partners and make appropriate synaptic contacts. The circuits that define spinal reflex arcs mediate spinal motor responses to specific sensory input and exhibit remarkable synapse specificity within

the spinal cord. In the sensory-motor reflex arc of the cutaneous maximus (Cm) muscle, chemorepulsive signaling mediated by Sema3E-plexin-D1 is critical for preventing the formation of monosynaptic inputs between Cm proprioceptor sensory neurons and Cm motor neurons; numerous aberrant monosynaptic inputs occur in the absence of this signaling pathway [18]. However, even in the absence of Sema3E, Cm motor neurons still receive input only from Cm afferents and not triceps (Tri) muscle afferents (another spinal reflex circuit), and Cm afferents do not form ectopic contacts with Tri motor neurons. At lumbar spinal cord levels, Sema3E-plexinD1 signaling also acts to prevent the formation of aberrant monosynaptic connections between specific sensory afferents and motor neurons of functionally non-related muscles, thereby controlling monosynaptic sensory-motor innervation of appropriate motor neuron pools [19•].

Sema3E-PlexinD1 signaling is also a critical determinant of specific connectivity in the thalamostriatal “direct” basal ganglia pathway. Sema3E, which is expressed and secreted by the axons of thalamostriatal neurons, binds its receptor plexinD1, which is selectively expressed by the direct pathway medium spiny neurons in the striatum. Sema3E-plexinD1 repulsive signaling acts to constrain synapse formation between the thalamostriatal neurons and the striatal medium spiny neurons of the direct pathway [20•]. Taken together, Sema3E-plexinD1 signaling in the CNS mediates several critical aspects of cell type- and pathway-specific connectivity.

Semaphorins regulate excitatory and inhibitory synaptogenesis

Semaphorins function as regulators of synapse formation, independent from their roles in guiding axonal and dendritic projections. This was initially suggested in *Drosophila* by work showing that the transmembrane semaphorin Sema-1a is critical for the formation of the giant fiber central synapse in developing pupae [21]. In mammals, Sema3F–neuropilin-2 signaling alters the balance between excitation and inhibition in the mouse cerebral cortex [8] and in the hippocampus [22] by selectively constraining excitatory synapse formation. A similar role in hippocampal synaptic transmission has also been demonstrated for Sema3A [23]. The transmembrane semaphorin Sema5B reduces synapse number in cultured hippocampal neurons, although whether Sema5B exerts a similar effect *in vivo* remains unknown [24]. Other semaphorins facilitate excitatory synapse formation in cultured hippocampal neurons. The class 4 transmembrane semaphorin Sema4B promotes the formation of excitatory synapses in cultured hippocampal neurons [25] (Figure 1a). Interestingly, class 4 semaphorins, including Sema4B, can interact with PDZ domain-containing proteins such as PSD-95 when co-expressed *in vitro* [26,27,28], however the relevance of this interaction to synapse formation has not been determined.

Unlike excitatory synaptogenesis, the formation and maintenance of inhibitory synapses is much less well understood. Certain class 4 semaphorins are important regulators of inhibitory synaptogenesis. Sema4B promotes inhibitory synapse formation through effects on the maturation of postsynaptic specializations, similar to its role at excitatory synapses [25] (Figure 1b). Furthermore, the closely related Sema4D is enriched at synapses and exclusively promotes the assembly of inhibitory synapses in hippocampal neurons, requiring the plexin-B1 receptor [25,29•,30•] (Figure 1b). Interestingly, exogenously supplied

Sema4D *in vitro* suppresses epileptiform activity observed in organotypic hippocampal slices following TTX withdrawal. Taken together, these results show that Sema4D induces the formation of functional GABAergic synapses [29••].

Semaphorin-mediated synapse elimination

Though numerous signaling pathways regulate neural circuit assembly [31], these are not sufficient to prevent the formation of ectopic synapses [32]. To achieve precise nervous system wiring aberrant axonal tracts must be withdrawn, a process known as pruning, and excess synapses eliminated. Removal of aberrant synaptic specializations can occur along with, or independent of, axon pruning. Axonal trajectories destined to be pruned often are associated with pre- and postsynaptic specializations [33,34,35••]. For example, during the pruning of the mouse hippocampal infrapyramidal tract (IPT), a mossy fiber projection normally pruned postnatally by Sema3F [36], the *Rac* GTPase-activating protein (GAP) β 2-chimaerin inactivates Rac1 to induce pruning [35••]. IPT pruning is accompanied by the loss of presynaptic specializations formed earlier between the IPT axons and CA3 pyramidal dendrites. Interestingly, β 2-chimaerin-mediated signaling is not required for Sema3F-mediated dendritic spine constraint or repulsive axon guidance, demonstrating that different Sema3F/Nrp-2–PlexA3 signaling modalities are engaged to regulate distinct aspects of neural wiring.

Regulation of semaphorin signaling by neuronal activity

During early stages of neural circuit maturation, neurons can simultaneously respond to guidance cues and patterns of activity [37,38]. Therefore, electrical and guidance cue stimuli might converge to regulate synaptogenesis. This idea was addressed *in vitro* by showing that electrical activity modulates neuronal responses to chemotropic molecules [39,40]. A recent study demonstrates an *in vivo* role for such modulation at the *Drosophila* neuromuscular junction (NMJ) [41••]. Embryonic and larval body wall muscles secrete Sema-2a, a chemorepellent that signals through the Plexin B receptor to regulate motor axon guidance and neuromuscular connectivity [42]. Electrical activity in PlexB-expressing motor neurons enhances motor neuron responses to the muscle-derived Sema-2a, and this modulation of Sema-2a signaling requires an increase in calcium entry at the presynaptic terminal. Calcium entry in turn activates CaMKII, likely leading to the phosphorylation of downstream targets and subsequent increased sensitivity to Sema-2a. Genetic interaction experiments reveal numerous ectopic motor axon-muscle contacts in the context of reduced presynaptic activity and Sema-2a levels, showing that activity-induced enhancement of Sema-2a signaling in motor neurons is required for synaptic target selection [41••] (Figure 2). These results define a regulatory mechanism that exerts spatial and temporal control over chemorepulsion, demonstrating how molecular and electrical stimuli can be integrated to precisely coordinate synapse refinement *in vivo*.

The expression patterns and functions of several semaphorins in the adult hippocampus [22] raise the possibility that these cues signal later in development, and even in the adult, to regulate synaptic strength in response to experience. This idea is supported by the demonstration that expression of neuropilin-2 (Nrp-2), a secreted semaphorin co-receptor, is influenced by the activity-regulated microRNA-188 (miR-188). In response to long-term

potentiation (LTP), miR-188 expression increases, resulting in the downregulation of Nrp-2 expression and subsequent abolition of Nrp-2–mediated inhibition of excitatory synapse formation and synaptic transmission [43•]. It will be important to assess this mode of activity-dependent regulation of semaphorin signaling *in vivo*. These results suggest that Sema3F–Nrp-2 signaling, and possibly other semaphorin signaling pathways, are also regulated similarly in response to external stimuli [44].

Conclusions

Semaphorins join an ever-growing list of synaptogenic and synapse-limiting molecules, and they have emerged as important regulators of excitatory and inhibitory synaptogenesis and function. This is of particular interest since the molecular mechanisms underlying inhibitory synapse formation are poorly defined, and it suggests semaphorins participate in regulating network properties by modulating the balance between excitatory and inhibitory synaptic functions.

Semaphorin influences on synapse formation and function raise the possibility that these molecular cues are involved in the pathogenesis of disorders characterized by profound defects in synaptic transmission, including epilepsy and various cognitive disorders. Indeed, Sema3F and Sema4D influence neuronal activity in mouse epilepsy models [22,29••], suggesting potential targets for the development of novel therapeutic approaches directed toward ameliorating epilepsy symptoms. Since electrical activity can modulate semaphorin signaling *in vivo*, integration of semaphorin signaling and neuronal activity may provide for precise control of neuronal morphology, synaptogenesis, and plasticity throughout life.

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Highlights

- Semaphorins affect the spatial distribution of synapses by regulating dendritic arborization.
- Semaphorins directly affect the formation of excitatory and inhibitory synapses.
- Sema3E-PlexinD1 signaling is involved in pathway-specific CNS connectivity.
- Semaphorins mediate synapse refinement during the pruning of exuberant neuronal connections.
- Electrical activity can modulate neuronal responses to semaphorins during synapse refinement.

a. Semaphorins in excitatory synapse formation

b. Semaphorins in GABAergic synapse assembly

Figure 1. Semaphorins directly affect synapse formation, altering the balance between excitation and inhibition

(a) Semaphorins and excitatory synaptogenesis. (I) Sema3F inhibits excitatory synapse formation by constraining the density of dendritic spines along apical dendrites of deep layer cortical pyramidal neurons. (II) Sema4B promotes the assembly of glutamatergic synapses in cultured hippocampal neurons. The Sema4B cytoplasmic domain includes a PDZ-binding motif that can associate with PSD-95, possibly mediating assembly of postsynaptic specializations (though this has yet to be demonstrated).

(b) Semaphorins and the assembly of inhibitory synapses. (I) Sema4D promotes GABAergic inhibitory synaptogenesis in hippocampal neurons *in vitro* and *in vivo*. Sema4D is required in postsynaptic neurons, where it is mainly localized to synapses, both *in vitro* and *in vivo*. However, plexin-B1 could act *in cis* (in the postsynaptic neuron) or *in trans* (in the presynaptic terminal) to mediate the assembly of inhibitory synapses. Posttranslational proteolytic Sema4D processing occurs *in vitro* and *in vivo* but apparently is not required for Sema4D regulation of GABAergic synapse assembly. (II) Sema4B promotes the assembly of inhibitory synapses by affecting postsynaptic specializations.

Figure 2. Electrical activity and chemotropism in synapse refinement *in vivo*

(a) In *Drosophila* embryos and larvae, electrical activity in motor neurons renders them more sensitive to the muscle-derived chemorepellent Sema-2a, causing exuberant motor neuron terminals to withdraw and preventing aberrant synapse formation at the *Drosophila* neuromuscular junction.

(b) Null mutations in *Sema2a* or (c) ion channels, allow motor neurons to establish ectopic synaptic contacts on somatic muscles that mature into functional synapses.

(d) Electrical activity in motor neurons synergizes with the response to Sema2a, mediated by the plexin B receptor (expressed in motor neurons), and regulates formation of

neuromuscular junctions. A partial loss of presynaptic activity accompanied by a partial loss of chemorepulsion leads to the formation of ectopic synaptic contacts on muscles. Therefore, electrical activity interacts with Sema-2a–Plexin-B chemorepulsive signaling to increase motor neuron responses to Sema-2a.