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The coordinate regulation of sensory afferent CNS targeting and CNS longitudinal tract organization in *Drosophila* during neural development

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Introduction

Sensory neurons translate a rich diversity of peripheral stimuli into select neuronal activities. These neural signals, representing different modalities and locations of sensory inputs, are delivered to distinct CNS regions in both vertebrates and invertebrates. Within each region of the nerve cord, longitudinal neural tracts provide connections between body segments and the brain, serving to exchange and integrate information along the anterior-posterior axis. Recent studies raise the possibility that both the establishment of CNS longitudinal tracts and specific sensory afferent input to these same regions are coordinately regulated so as to mediate correct processing of corresponding sensory information (Rajagopalan et al., 2000; Simpson et al., 2000; Zlatić et al., 2003; Wu et al., 2011). We address this issue here using the embryonic and larval nervous systems of *Drosophila*. Though our colleague Jack Griffon does not work on this model system, his appreciation for the phylogenetically conserved cellular and molecular mechanisms that elaborate sensory neuron connectivity during neural development provides us with constant encouragement in this and our other ongoing work. It is our hope that this present study (Wu et al., 2011), reviewed below, will provide new ways to think about sensory neuron connectivity and how it might be modulated in the human nervous system to address clinical issues related to neuronal degeneration and regeneration.

Semaphorin-plexin signaling functions together with Slit-Robo guidance to ensure precise assembly of CNS longitudinal neural tracts

Longitudinal axonal projections are a major component of the nerve cord in both vertebrates and invertebrates. How these individual tracts are organized during development is an intriguing question. To form each longitudinal pathway, growing axons need to cross segmental boundaries and recognize related axons, from among a large number of distinct neuronal cell types in the next adjacent segment, with which to form a continuous connection. Classical cellular experiments using invertebrate models demonstrate that interactions between pioneer axons are important for the establishment of continuous longitudinal pathways (Goodman et al., 1984). However, the molecular mechanisms underlying axon-axon recognition in this context are not fully understood.

Semaphorin guidance cues signaling through their plexin receptors have been implicated in CNS longitudinal tract formation. There are only two plexin receptors in *Drosophila* (as opposed to nine in mammals), Plexin A (PlexA) and Plexin B (PlexB), and they mediate

distinct functions for proper development of select CNS longitudinal tracts. For the three major longitudinal tracts labeled by a monoclonal antibody (1D4 MAb) directed against the Fasciclin II protein in the *Drosophila* embryonic CNS (Fig. 1A), PlexA is required for the organization of the most lateral of these pathways, while PlexB is specifically required for the intermediate pathway (Winberg et al., 1998; Ayoob et al., 2006). Transmembrane semaphorin-1a (Sema-1a), signaling through PlexA, is also required for the lateral 1D4⁺ tract formation (Yu et al., 1998). The two secreted semaphorins in *Drosophila*, semaphorin-2a (Sema-2a) and semaphorin-2b (Sema-2b), are both PlexB ligands that mediate important CNS functions (Wu et al., 2011).

Sema-2b is expressed by a small population of longitudinally projecting axons during the CNS development. In the present study (Wu et al., 2011), we find that Sema-2b signals attraction through the PlexB receptor to promote selective fasciculation of these Sema-2b-expressing axons in a cell-type-autonomous manner, thus establishing an early forming longitudinal pathway in the intermediate region of the CNS. By presenting Sema-2b on its surface, this pioneer pathway also serves as a scaffold to organize other later forming adjacent longitudinal projections in the same CNS region, including the intermediate 1D4⁺ longitudinal tract.

The other PlexB ligand, Sema-2a, is more broadly expressed and enriched in the medial region of the developing CNS. In contrast to Sema-2b, Sema-2a mediates axonal repulsion to ensure proper lateral positioning of CNS longitudinal tracts. Both Sema-2a and Sema-2b are instructive guidance cues. Sema-2a-mediated repulsion and Sema-2b-mediated attraction act in concert through the same PlexB receptor, thus assuring proper organization of select CNS longitudinal tracts.

These results not only provide the first evidence for how semaphorin guidance cues regulate the assembly of these inter-segmental longitudinal connectives, but they also reveal how sequential functions of distinct neuronal guidance systems establish and then consolidate basic neuropil structures in the developing CNS. The three CNS longitudinal regions in *Drosophila* are established in order, from medial to lateral, through the control of the Slit-Robo signaling cascade. The repellent Slit is expressed from the ventral midline to establish a repulsive gradient toward the lateral regions of the CNS. Differential expression of Robo receptors specifies the initial lateral positions for longitudinally projecting axons (Dickson and Zou, 2010). Within the intermediate CNS region, Sema-2b-PlexB-mediated signaling serves to promote selective fasciculation of individual axons into a tightly organized longitudinal pathway, thus ensuring the fidelity of Slit-Robo encoded lateral positional information. Although the exact cellular mechanisms remain to be determined, Sema-1a-PlexA signaling specifically mediates the proper formation of the most lateral pathway (Yu et al., 1998). Together, Slit-Robo and semaphorin-plexin guidance signaling cascades function in tandem, with Slit-Robo specifying long-range positioning of longitudinal connections, and Sema2b-PlexB instructing short-range local fasciculation. Additional cues, including cell surface adhesion molecules, provide further refinement of the local axonal interactions within each restricted region of the developing neuropil to establish the correct patterning of these axons during neural development (Lin et al., 1994).

Semaphorin-plexin and Slit-Robo signaling also function together to mediate precise sensory afferent targeting to the CNS

In *Drosophila*, different classes of sensory afferents innervate distinct CNS regions during development (Merritt and Whittington, 1995). Thus, CNS longitudinal pathways receive select sensory input required for select neural functions. The same Robo code that is important for determining the lateral positions of CNS longitudinal axons also regulates the

medio-lateral targeting of sensory axons in the developing CNS neuropil (Zlatic et al., 2003). Both *Sema-1a* and *Sema-2a* further restrict sensory afferent innervation to specific CNS regions, while *Sema-2b* functions to promote selective targeting of sensory afferents to their appropriate central targets.

Chordotonal (ch) sensory neurons mediate mechanosensation in *Drosophila* (Eberl, 1999). The axonal terminations of ch sensory afferents are initially confined to the CNS intermediate region under the influence of the *Slit-Robo* code (Zlatic et al., 2003). Then, *PlexB*-mediated guidance controls the selective targeting, branching, and fasciculation of ch axons along the intermediate *1D4⁺* longitudinal CNS tract. *Sema-2b*-mediated attraction guides ch sensory terminals to their proper target region, however, we find that *Sema-2a*-mediated repulsion prevents ectopic innervation (Fig. 1B and 1C). Loss of *Sema-2b*, or selective disruption of *PlexB* signaling in ch axons, severely compromises normal ch afferent targeting in the CNS (Fig. 1C'), and this results in specific larval deficits in behaviors normally mediated by ch neurons (Wu et al., 2011). This shows that precise ch afferent connections to their appropriate CNS partners is required for central processing of ch sensory information, and also for the subsequent output consisting of appropriate larval behavioral responses to sensory stimuli.

Therefore, in this study (Wu et al., 2011), we find that the same guidance systems critical for organizing interneurons that reside in the intermediate longitudinal pathways are also deployed sequentially to generate precise ch sensory input onto these same CNS neurons. These two processes are intrinsically linked so as to facilitate the accurate connection of related CNS structures belonging to the same functional neural circuit.

Common molecular mechanisms underlying sensory circuit organization in both vertebrates and invertebrates

Midline expression of the *Slit* repellent is a common feature in both the *Drosophila* ventral nerve cord and the vertebrate spinal cord. *Slit-Robo* signaling has also been shown to regulate CNS longitudinal tract formation in vertebrates (Farmer et al., 2008). Similar functions of repulsive semaphorin guidance cues critical for the restriction of sensory afferent projections to distinct CNS targets have also been observed in the mouse. The related secreted semaphorin *Sema3e* acts through *plexin D1* to ensure the specificity of murine proprioceptive sensory-motor circuitry in the ventral spinal cord through repellent signaling (Pecho-Vrieseling et al., 2009). In addition, repulsive signals from the transmembrane semaphorins *Sema-6C* and *6D* in the dorsal spinal cord ensure appropriate murine proprioceptive sensory afferent trajectories in the dorsal spinal cord (Yoshida et al., 2006). However, very little is known about possible attractive signals that might serve to promote selective sensory afferent targeting in vertebrates, and the exact mechanisms underlying vertebrate CNS longitudinal tract formation remain to be determined. It is likely that a phylogenetically conserved ensemble of short-range cues functions together with long-range cues to mediate both global and local organization of individual pathways.

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Figure 1. PlexB-mediated signaling is required for proper chordotonal sensory afferent innervation within the embryonic *Drosophila* CNS

(**A** and **B**) Wild-type late stage 16 *Drosophila* embryo stained with 1D4 (**A**; magenta) or anti-GFP (**B**; green), then filleted from the dorsal side to generate a flat-mount preparation. Anterior is to the left. The yellow box indicates the ventral nerve cord (VNC) region. (**A**) 1D4 staining reveals all peripheral motor axon projections and three longitudinally projecting CNS axon tracts on both sides of the VNC. (**B**) The *iav-GAL4* driver directs *UAS:syt-GFP* expression in chordotonal (ch) sensory neurons, which preferentially labels terminal branches of their afferent innervations to the CNS. (**C** and **C'**) CNS regions double labeled with 1D4 and anti-GFP are shown in higher magnification. (**C**) Ch axons normally elaborate their characteristic regular and continuous CNS innervation pattern along the

intermediate 1D4⁺ longitudinal tract in a wild-type background. (C') When PlexB signaling is specifically disrupted in ch neurons, ch afferent terminal branches are severely disorganized in the CNS. l, lateral; i, intermediate; m, medial.