and virtual-body-immersion could ultimately guide the development of applications where surrogate bodies can be used for navigating the world and interacting with others.

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Cracking the Combinatorial Semaphorin Code

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In this issue of *Neuron*, Wu et al. describe a combinatorial code of repulsive Sema-2a and attractive Sema-2b signaling that mediates mechanosensory axonal guidance, fasciculation, and synaptic target selection within the CNS of *Drosophila*. Their work exemplifies how a detailed, multilevel molecular-genetic analysis (from molecules to behavior) provides fundamental insights into neural circuit development.

One of the challenges facing developmental neurobiology is to understand how axons find their way through the developing embryonic landscape to establish functional neural circuits. Progress in understanding the mechanisms governing guidance and connectivity came with the discovery of chemotropic ligands and their receptors, molecules that include the Netrins, Cadherins, Semaphorins, Ephrins, and a host of Ig superfamily proteins. These chemotropic agents are either attractive or repulsive, in some cases acting over long distances via diffusible topographic gradients, and in other cases acting through direct contact (Kolodkin and Tessier-Lavigne, 2011). Not surprisingly, chemotropism is complex: the same ligand can be either attractive or repulsive depending on the receptor complexes expressed by the growth cone. Axons, in turn, usually express several guidance receptors. The combination of multiple guidance cues and receptors effectively constitutes a combinatorial "guidance code" that defines how an axon (or dendrite) will find its way. An intriguing hypothesis is that synaptic partners might share similar guidance codes, ensuring that their processes meet at specific locations within the developing nervous system, as a first step in forming a neural circuit.

In this issue of *Neuron*, Wu et al. (2011) provide compelling evidence to support this hypothesis. They show that a combinatorial code involving Semaphorins and their Plexin receptors guides the construction of a central neural circuit in the *Drosophila* embryo, involving sensory neurons and their interneuronal partners. The developing *Drosophila* CNS expresses three Semaphorins, a transmembrane Sema-1a protein that signals through the Plexin A (PlexA) receptor, and the secreted Sema-2a and -2b proteins. While Sema-2a was known to act as a chemorepellant, signaling through the Plexin B receptor (PlexB; Ayoob et al., 2006), much less was known about either Sema-2b or its receptor.

Sensory innervation of the embryonic CNS is perhaps less well known than other model systems in *Drosophila*, such as the eye, CNS midline, olfactory neuropil, or neuromuscular junction. Nevertheless, this paper shows it to be enormously powerful. The authors examined a group of mechanosensory neurons called chordotonal (ch) cells, that are born in the periphery and whose axons grow along peripheral nerves to innervate the CNS. Once there, the axons are faced with the daunting challenge of identifying the correct tracts to lead them to their synaptic partners. In the CNS, they find that the roadways are still under construction, with axon tracts and fascicles actively being established. Wu et al. (2011) show that the ch neurons and their interneuron partners use the same molecular guidance system to rendezvous at a specific site within the developing ventral nerve

cord (VNC, akin to the vertebrate spinal cord).

The embryonic and larval VNC is organized as a latticework of longitudinal axon tracts that link the local segment-specific circuits together, and transverse tracts that enable communication between the left and right hemisegments. A subset of the longitudinal axon tracts can be selectively labeled by virtue of their expression of the NCAM homolog Fasciclin2 (Fas2). This IgCAM is expressed by the axons of three parallel longitudinal tracts, known as the medial, intermediate, and lateral bundles, located on either side of the midline. Work by the Goodman lab (UC Berkeley) and the Dickson lab (Vienna, Austria) had shown that the spacing of these bundles is due to various degrees of chemorepulsion by Slit, a protein secreted by glial cells at the midline. The responsiveness of longitudinal axons to Slit depends on which subset of the three Roundabout (Robo) receptor proteins the neuron expresses (Rajagopalan et al., 2000; Simpson et al., 2000). This "Robo code" was an early demonstration of how combinatorial signaling can organize a developing nervous system.

In *Drosophila*, each class of sensory neuron sends axons that terminate at a specific region within the developing VNC. The Bate lab (Cambridge, UK) discovered that sensory axons that project along the mediolateral axis of the VNC use Robo to respond to the same-midline-derived gradient of Slit that organizes the longitudinal tracts (Zlatic et al., 2003). To gain their correct position along the dorsoventral axis, however, they are guided by a Plexin-mediated response to gradients of Semaphorins (Zlatic et al., 2009). Thus, the three-dimensional topography of sensory neuron projections is mediated by two orthogonally oriented chemorepulsive gradients. Zlatic et al. (2009) also showed that mechanosensory axons from the chordotonal organ preferentially grow toward and selectively fasciculate with the intermediate Fas2-expressing tract. Homophilic Fas2 adhesion cannot account for this specificity, however, as the ch axons are not themselves Fas2 positive. Instead, ch axons are guided to that location by Semaphorin signaling. Consistent with this observation, only the intermediate tract and the ch axon projections are disrupted in PlexB mutants.

Wu et al. (2011) have extended this result at the cellular and molecular level, demonstrating that guidance depends on the coordinate response of the ch neurons and the intermediate fascicle axons to the two secreted semaphorins, Sema-2a and Sema-2b. Both Sema-2a and Sema-2b have been proposed as ligands for the PlexB receptor. The authors show convincingly that both molecules do indeed signal in this system through PlexB: mutants of either semaphorin gene alone do not fully recapitulate the PlexB phenotype, but double mutants do. The authors further demonstrate that the two Semaphorins have opposite effects in this system. Sema-2b acts as an attractive cue while Sema-2a acts as a chemorepellant. Together, they confine ch axons and their targets to the correct neuropilar region.

The authors began to crack the combinatorial Sema-2 code by examining the complexities of Sema-2-PlexB signaling. In particular, Sema-2b expression is strongest on the intermediate tract, and fasciculation phenotypes were rescued by selective expression of either normal or a membrane-tethered Sema-2b in those cells. This shows that Sema-2b both attracts innervating axons and enhances fasciculation over a short range, perhaps through direct contact. Moreover, Sema-2b attraction is required within two populations of neurons that use the intermediate tract for guidance, as a transmembrane version of Sema-2b lacking its cytoplasmic domain disrupted the axonal guidance of both ch and MP1 neurons, whose axons pioneer the intermediate tract (Hidalgo and Brand, 1997). Sema-2a, however, is expressed more widely, deflecting axons from inappropriate regions through chemorepulsion, a mechanism that might corral errant axons and guide them back to their correct destination. The complementary actions of a short-range attractive cue amidst a long-range, diffusible repellant are reminiscent of two other axon guidance systems in *Drosophila*. In the embryonic CNS, commissural axons approach the midline through Netrin-mediated chemoattraction but depend on Slit repulsion to prevent recrossing (Yang et al., 2009). At the developing neuromuscular junction, motoneuron axons seek out specific muscle fibers through chemoattraction but depend on Sema-2a repulsion to prune off-target contacts (Carrillo et al., 2010).

Wu et al. (2011) also examined the behavioral consequences of having mistargeted ch axons. The chordotonal organ is responsible for specific forms of mechanosensation in the larva. When normal larvae are exposed to high frequency vibrations, they slow down and exhibit a characteristic head turning behavior. This behavior is absent in animals lacking functional ch organs. Larvae whose ch axons fail to recognize the intermediate tract and therefore grow to an inappropriate location also fail to respond to high frequency vibration. It would be interesting to determine whether the misdirected ch axons now establish novel synapses at their ectopic locations, perhaps causing vibration to drive unrelated sensory circuits and behaviors, a form of *Drosophila* synesthesia.

One of the fascinating questions to arise from this study is how Sema-2a and Sema-2b, proteins with 68% sequence identity, can mediate opponent repulsive and attractive responses through the same PlexB receptor. One plausible explanation is that there are one or more coreceptors that form PlexB complexes to mediate the specific repulsive or attractive behavior. However, the most likely candidate for a PlexB coreceptor, Off-Track, was ruled out by the authors. A major challenge will be to resolve the molecular mechanisms that govern these distinct responses, as well as to determine how downstream PlexB signaling affects the cytoskeleton in such dramatically different ways.

The deeper question is whether the model for guidance revealed by this study is a general one or just a specific detail for one class of sensory neuron projections and their partners. It would be intriguing if a combinatorial coding regime, perhaps utilizing other guidance molecules as well, is used to guide synaptic partners to specific rendezvous sites within the CNS, as a first step in forming neural circuits. If so, this would help explain how the fundamental topographic gradients of the CNS are used to define the locations and assembly of specific neural circuits.

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