PERSPECTIVES: DEVELOPMENTAL NEUROSCIENCE

Moving On

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It is a feeling that many travelers will know: No sooner has a particular destination been reached, than it loses its charm and one begins to long for the next. For the traveler, this may make for an unsettling journey. But for axons navigating throughout the developing embryo in search of their synaptic partners, such restlessness is essential to keep them moving on from one intermediate target to the next, until they reach their final destination. This restlessness does, however, pose something of a paradox. How can an axon first be attracted to an intermediate target, but then lose interest as soon as it arrives, moving on to more enticing targets elsewhere? On page 1928 of this issue, Stein and Tessier-Lavigne (*1*) offer a surprising solution to this mystery.

For commissural axons—those axons that connect the two symmetrical halves of the central nervous system (CNS)—the midline of the body is a critical intermediate target. These axons grow first toward the midline, but when they reach it they then continue growing, moving across the midline into the opposite half of the body. Studies of axon outgrowth in mammalian CNS explant cultures have shown that commissural axons are directed by guidance molecules produced by a specialized group of midline cells that form a structure known as the floor plate. These guidance molecules include netrin, an attractant (*2, 3*), and Slit, a repellent (*4, 5*). Remarkably, commissural axons switch their responsiveness to these cues as they cross the midline (see the figure). Before crossing, they are attracted by netrin (*2, 3*) but insensitive to Slit (*4, 5*), whereas after crossing they no longer respond to netrin (at least in the hindbrain) (*6*) and are now repelled by Slit (*5*).

Switching sensitivity to these guidance cues seems like a good way to keep commissural axons moving through the midline, but how is attraction at the midline turned off and repulsion turned on? In *Drosophila*, where the activity of Slit in axon guidance was first described (*7*), commissural axons increase their expression of the Slit receptor Robo as soon as they cross the midline (*8*). This explains how commissural axons acquire sensitivity to Slit. But how do they lose their sensitivity to netrin?

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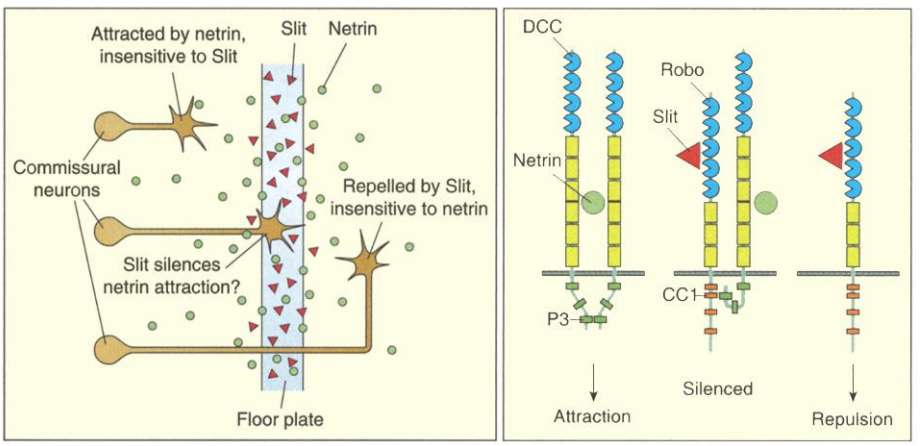
Enter Stein and Tessier-Lavigne (*1*) with their elegant study using the *Xenopus* spinal neuron turning assay pioneered by Poo and colleagues (*9*). In this assay, an isolated spinal axon growing in vitro is exposed to a gradient of a purified guidance factor released from a micropipette, and its growth rate and turning response are then monitored with time-lapse microscopy. This assay offers two important advantages over the more traditional explant assays. First, one can assess the response of a single axon to a single guidance cue. Second, by injecting messenger RNA into the blastomeres of early embryos, these axons can be forced to express any desired guidance receptor.

Stein and Tessier-Lavigne first noted that spinal axons from stage 22 *Xenopus* embryos are attracted by netrin (*10*) and insensitive to Slit, whereas stage 28 neurons are insensitive to netrin but repelled by Slit. They next asked what would happen if stage 22 axons were exposed to both signals simultaneously, as is the case for commissural axons in vivo. Surprisingly, when confronted with the two cues together, these axons were neither attracted nor repelled! This cannot be just a case of repulsion and attraction canceling each other out, because these axons are not repelled by Slit at all, and Slit does not block the turning response to a different attractant. Somehow, Slit specifically silences the ability of netrin to attract axons.

In a tour de force study with chimeric receptors and heterologous ligands in the *Xenopus* assay, as well as coimmunoprecipitation experiments with transfected cells, Stein and Tessier-Lavigne (*1*) go on to show that this silencing effect depends on a direct interaction between the cytoplasmic domains of Robo and the netrin receptor, DCC. This interaction is mediated by short conserved domains in each receptor—CC1 in Robo and P3 in DCC (see the figure). In a dramatic conclusion to their experiments, Stein and Tessier-Lavigne were able to reconstitute both the physical association and the silencing effect with a pair of chimeric receptors that were activated by two completely different ligands and associated through different interaction domains.



Axons in transit. (Left) Commissural axons cross the floor plate, an intermediate target, as they travel to their final destination on the opposite side of the body. As they cross the midline, these axons switch their responsiveness to the guidance cues netrin and Slit, produced by cells in the floor plate. As axons reach the floor plate, Slit may silence attraction by netrin (*1*). **(Right)** Changing interactions between DCC (the netrin receptor) and Robo (the Slit receptor) may alter the responsiveness of commissural axons to netrin and Slit guidance cues, thus ensuring that they continue on toward their final destination.

In a second paper on page 1976 of this issue (*11*), Stein and colleagues use a similar series of assays to show that attraction by netrin depends on the self-association of DCC receptors through the same P3 domain. They also make a compelling case that, at least for *Xenopus* spinal axons and mammalian commissural axons, netrin signaling does not require the adenosine A2B receptor, as recently suggested (*12*). Previously, Stein and co-workers demonstrated that DCC can also mediate repulsion by forming a complex with another netrin receptor called UNC5 (*13*). In this case, the interaction depends on a different motif in the DCC cytoplasmic domain called P1. DCC can thus be switched from attraction to repulsion, or silenced completely, depending on its binding partner. The mechanism appears to be similar in each case—binding of ligand to a coreceptor (netrin to DCC for attraction, netrin to UNC5 for re-

pulsion, and Slit to Robo for silencing) allows the cytoplasmic domain of this coreceptor to bind to the cytoplasmic domain of DCC. Presumably, a different set of intracellular signaling proteins is then recruited to each receptor complex.

These new studies raise a number of important questions. Clearly, the most urgent need is to find out whether this silencing mechanism also operates in commissural axons in vivo. Fortunately, among their large collection of mutant and chimeric receptors, Stein and Tessier-Lavigne have generated a form of DCC that still mediates attraction by netrin but cannot be silenced by Slit. Coaxing commissural axons to express this unsilenceable receptor in vivo should reveal what role, if any, silencing might play in forcing axons to move on once they have reached the midline.

If, as seems likely, silencing does indeed occur in vivo, then it will also be important to figure out how commissural axons regulate their response to Slit—perceiving it first as a silencer, then as a repellent, and later perhaps even as a branching and elongation factor (*5, 14*). Regulation of Robo can only be part of the answer. In particular, Robo regulation cannot explain the different responses to Slit observed in stage 22 and stage 28 *Xenopus* spinal neurons. Perhaps commissural neurons change their responses to Slit according to some intrinsic program, in much the same way that they also change their neurotrophic requirements as they complete each leg of their journey (*15*).

The discovery of this silencing phenomenon also suggests an alternative explanation for the midline guidance errors observed in *slit* and *robo* fly embryo mutants. Could it be that axons stray across or linger at the midline in these mutants in part because of a failure to silence attraction, rather than simply because of a loss of midline repulsion as previously thought? Separating the silencing and repellent functions of Slit would help to resolve this issue. Stein and Tessier-Lavigne suggest a way to do just this: In the *Xenopus* assays, a mutant form of Robo that lacks the CC1 domain can still mediate repulsion but is unable to silence attraction. This mutant form of Robo has already been expressed in flies, and results in a low frequency of midline crossing errors (*16*). It is important to note, however, that *Drosophila* has two additional Slit receptors, Robo2 and Robo3 (*17, 18*). Both receptors contain the CC1 domain and so may also contribute to silencing. It will be interesting to see whether deleting the CC1 domains of all three Robo receptors leads to more severe midline crossing errors and, if so, whether these defects require netrin and DCC activity as predicted by the silencing model.

Finally, the studies of the Tessier-Lavigne laboratory force us to revise our view of how axons respond to multiple guidance cues. In vivo, axons are simultaneously exposed to a number of different attractive and repulsive forces. It has generally been thought that the axon integrates all of these signals in order to calculate its next move (*19*). But, as Stein and Tessier-Lavigne show, multiple guidance signals can also be combined in a hierarchical fashion, with one signal silencing the response to another. These two guidance strategies each make sense in different contexts. Integration, for example, has been most clearly demonstrated in the selection of different muscle targets by motor axons in *Drosophila* (*20*). Here, subtle differences in the way each axon responds to various muscle attractants and repellents may be an effective way to bias their preferences for specific muscle targets. In contrast, hierarchical guidance may be the better strategy at intermediate targets where axons must suddenly and drastically switch their preferences to ensure that they keep moving on toward their final destination.

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PERSPECTIVES: LASER PHYSICS

Toward Attosecond Pulses

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In which scientific discipline would you expect to find the fastest recorded events? Surprisingly, the answer is not electronics, or even atomic physics, but optics. In the last decade, laser science has succeeded in generating pulses of light lasting less than 10 femtoseconds (fs), where 1 fs = 10^{-15} seconds. Femtosecond pulsed lasers have provided unprecedented insights into molecular processes such as reaction dynamics. So far, most research has concentrated on pulses in the visible or near-infrared part of the light spectrum. At these wavelengths, the optical wave takes about 3 fs to complete one oscillation, so that state-of-the-art 5-fs laser pulses correspond to less than two optical cycles. Optical pulses much shorter than this require substantial effort to produce, and, because light propagates as an oscillating electromagnetic wave, they are fundamentally limited to the "single-cycle" duration of about 3 fs.

Now, a new area of experimental physics is emerging, one sufficiently radical to be defined by its own prefix—attosecond science. By using femtosecond optical pulses to generate wavepackets in the soft x-ray region, where wave cycles last for only about 50 attoseconds (as) or 50×10^{-18} seconds, it should be possible to produce multicycle x-ray pulses with subfemtosecond durations. In this issue, Drescher *et al.* (*1*) report a first step in this direction. The authors have both created and measured x-ray pulses with durations below the carrier wave period of the original optical pulse.

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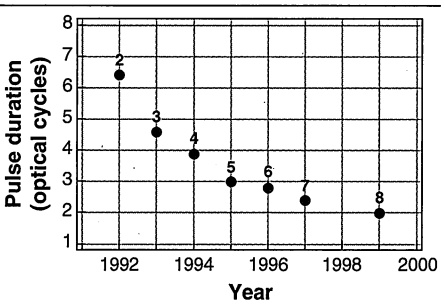

Reaching a plateau. The steady decrease in the duration of optical pulses produced by femtosecond lasers over recent years has begun to slow down, implying that new approaches will be needed to produce subfemtosecond wave packets. The "single-cycle" limit for typical optical pulses has a duration of 2.5 to 3 fs and corresponds to the time taken for the propagating optical wave to complete one full oscillation. Numbers in the figure correspond to reference numbers.

Molecules are characterized by bending and stretching motions with time periods lasting several tens of femtoseconds or more, and femtosecond pulses can be used to study these vibrational motions. Measure-