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Axon Guidance: FLRTing Promotes Attraction

A recent study demonstrates a new mechanism by which crosstalk between multiple guidance cues is integrated during axon pathfinding. FLRT3 is a novel co-receptor for Robo1 that acts as a context-dependent modulator of Netrin-1 attraction in thalamocortical axons.

Laura Anne Lowery

One of the most remarkable feats of early neural development occurs when neurons send out an axon to navigate through the embryo, eventually forming intricate networks of connections that are essential for all subsequent neural function. At the tip of each axon sits the growth cone (Figure 1), the dynamic hand-like structure that steers through the complicated, ever-changing embryonic landscape and interprets guidance cues in order to find and connect with its final target [1]. Many decades of axon guidance research have defined key extracellular cues, receptors and signaling pathways that are essential for guiding growth cones to their destinations [2,3]. Yet, we still do not understand the logic of how simultaneous inputs of numerous axon guidance cues are interpreted to steer the growth cone in the right direction (Figure 1). A new study in this issue of *Current Biology* by Leyva-Diaz *et al.* [4] provides insights into this important question by demonstrating a mechanism through which crosstalk between multiple cues is integrated during the guidance of thalamocortical axons.

The thalamocortical projection (neurons sending axons from the thalamus to the neocortex) is responsible for a significant component of higher-level processing in the mammalian brain, including the sensory pathways of vision, hearing, and touch. It represents a powerful and complex model system for examining the mechanisms that regulate the

precise positioning of axonal tracts [5,6]. Neuronal cell bodies are spatially organized within the thalamus, with axons extending and then spreading out to acquire a precise rostrocaudal position before arriving at the neocortex (Figure 2A). Rostral nuclei project to the rostral motor cortex, while caudal nuclei project to the caudal visual cortex [6,7]. How this topographic positioning is achieved has been an area of intense investigation in the axon guidance field. While the repertoire of axon guidance factors known to be involved in steering thalamocortical axons includes the expected cast of chemotropic factors — Netrins and Slits, as well as Ephrins and Semaphorins [5,6] — the recent work from the lab of Lopez-Bendito [4] adds a new player to the team, the fibronectin and leucine-rich transmembrane protein FLRT3.

The study by Leyva-Diaz *et al.* [4] builds on previous work from Bielle *et al.* [8], which identified interesting interactions between Netrin and Slit that occur specifically in the rostral thalamocortical axons (rTCAs) [8]. For this particular axonal subset, when the guidance cue Slit1 is presented alone, it leads to a repulsive response, while the guidance factor Netrin-1 alone has no chemotactic effect. Yet, the two factors in combination attract rTCAs (Figure 2B). This finding was intriguing as the neighboring intermediate TCAs (iTCAs) are not attracted to Netrin-1 with or without Slit1, despite the fact that both rTCAs and iTCAs express the same Netrin receptors, including DCC

(Deleted in Colorectal Cancer), as well as receptors for Slit1. It was unclear what distinguished the behaviors of these two subsets of axons given their molecular similarities.

In the present study, Leyva-Diaz *et al* [4] demonstrate that Netrin-1 attraction is activated specifically through Slit1/Robo1 signaling, which appears to promote DCC vesicular transport to the growth cone surface via PKA activation (Figure 2C). The increase in DCC at the surface could then explain the enhancement in Netrin-1 attraction. Thus, these new results further unravel the intracellular signals involved in rTCA pathfinding and provide a new framework for understanding how guidance cues can modulate the growth cone response. However, it was still a mystery why only the rTCAs were attracted to the Netrin-1/Slit1 combination, as both rTCAs and iTCAs express similar levels of Robo1. Which molecular component can account for the difference in attraction? The answer, it turns out, is FLRT3.
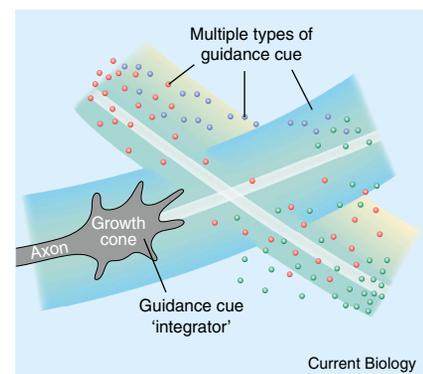

Figure 1. The growth cone steers through the embryo.

A simplified cartoon schematic depicting the complex landscape of guidance cues encountered by navigating growth cones during early neural development. The growth cone is responsible for integrating the numerous cues in order to form accurate and precise neuronal connections.

Leyva-Diaz et al. [4] noted that FLRT3 was a particularly intriguing candidate to examine in the context of Netrin-1 signaling in rTCAs. Not only is FLRT3 known to regulate neurite outgrowth [9,10], it was recently shown to bind the Netrin receptor Unc5 [11], which plays a role in caudal TCA guidance [12]. The authors discovered that FLRT3 was expressed in a decreasing rostral-to-caudal gradient in developing thalamic neurons; hence, it was enriched specifically in rTCAs. Strikingly, the authors identified Robo1 as a FLRT3 interactor. The FLRT3–Robo1 interaction in thalamic neurons was extensively validated, providing convincing evidence that FLRT3 is a novel co-receptor that associates with Robo1 and can modify Robo1 activity.

FLRT3 is indeed the missing piece necessary to mediate Slit1-induced Netrin-1 attraction, as lack of FLRT3 in rostral thalamic explants blocks Netrin-1 attraction, abolishes the increase in surface DCC, and prevents PKA activation. Compellingly, they demonstrate that ectopic expression of FLRT3 in iTCAs leads them to behave like rTCA neurons, becoming attracted to the Netrin-1/Slit combination and showing pathfinding responses of rTCAs in an *ex vivo* assay. Finally, they examine rTCA pathfinding *in vivo* and discover that FLRT3 knockdown leads to a loss of directional positioning of the rTCAs. Thus, FLRT3 is proposed to act as a context-dependent modulator of Netrin1/Slit1 signaling.

One of the most exciting aspects of this study is that it offers new conceptual insights into how multiple guidance cues and receptors can be coordinated to guide the growth cone. It has been known since the 1990s that growth cones can differentially respond to Netrin-1 depending on which guidance receptors are present on the growth cone surface [13]. Soon after that initial breakthrough, Stein and Tessier-Lavigne [14] showed that activation of the Slit receptor Robo can silence the attractive effect of Netrin-1 through Robo association with the Netrin receptor DCC. Conversely, Netrin-1 can also attenuate Slit-Robo repulsion in other situations [15]. However, both of these cases are examples of a hierarchical silencing mechanism, where one cue response suppresses the effect of another. In the new paper by Leyva-Diaz et al. [4],

we learn of a different type of interaction, in which a new response (attraction) emerges from two disparate ones (repulsive and neutral) with the assistance of a co-receptor (in this case, FLRT3). This may reflect a broadly used mechanism for context-dependent modulation of axon guidance behaviors.

An additional key point is that the authors reveal a possible novel mechanism for *how* the downstream signaling mechanisms occur to modify the growth cone response. While previous reports have suggested that the presence or absence of a co-receptor protein activates separate downstream signaling pathways, this study suggests that activation of a co-receptor (FLRT3) can change the surface expression of an additional guidance receptor (DCC) through promoting downstream signals (PKA activation). This secondary change in DCC surface expression then converts the response of the axons from neutral to attraction. This insight is particularly exciting as probing the regulation of vesicular trafficking in the growth cone in response to guidance cues is one of the vanguards in the axon guidance field. Thus, this work has opened up the possibility for specific questions to be addressed regarding the intracellular machinery that regulates DCC movement to the surface downstream of FLRT3 activation. In particular, how does Slit1 binding to the FLRT3–Robo1 complex lead to PKA activation, and how does this activation drive DCC transport to the membrane? Another remaining question is how does FLRT3 become activated in rTCAs? The authors report that FLRT3 does not bind to Netrin-1 nor Slit1, and thus, FLRT3 may be activated by an unknown ligand or may act independently of a direct ligand in this context.

This study nicely illustrates exactly the type of approaches that are required in order to further refine our understanding of the growth cone guidance mechanism. In particular, combining *in vitro* neural culturing with *in vivo* axon guidance analysis holds considerable potential for determining how multiple overlapping gradients of cues are interpreted during axon guidance, and future studies utilizing these multi-layered and combinatorial approaches will be necessary. While Leyva-Diaz et al. [4] takes us further along the journey
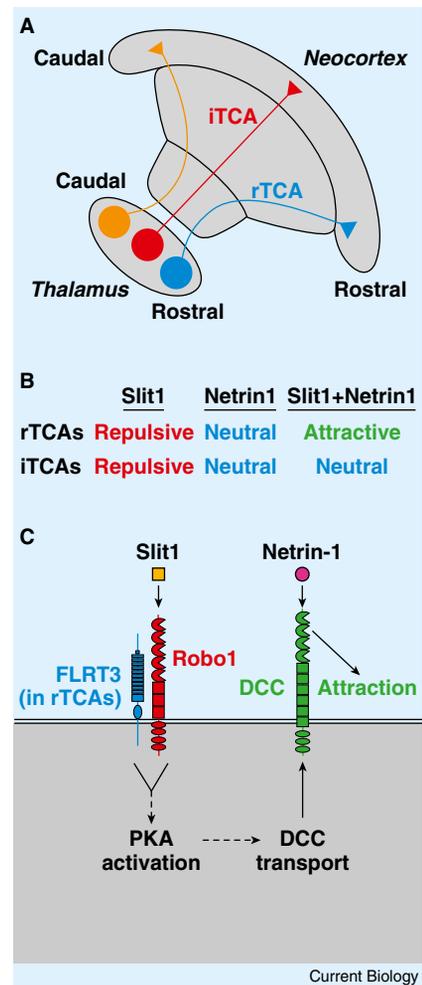

Figure 2. New insights for pathfinding of rostral thalamocortical axons.

(A) A cartoon schematic of the topographical organization of TCAs. Rostrally positioned thalamic nuclei project to the rostral motor cortex, while caudally-positioned thalamic nuclei correspondingly project to the caudal visual cortex. rTCA, rostral thalamocortical axon; iTCA, intermediate thalamocortical axon. Adapted from Bielle et al. [8]. (B) A summary of TCA chemotactic responses to Slit1 and Netrin-1 during *in vitro* neural culture assays. An attractive response only occurs when Slit1 and Netrin-1 are both presented to rTCAs. (C) Novel pathway of Slit1/Netrin-1 attractive response by rTCAs. FLRT3 modifies Robo1 function downstream of Slit1 signaling, leading to PKA activation, increased DCC transport to growth cone surface, and hence, attraction by Netrin-1.

towards understanding axon guidance cue integration, it is clear that there are many exciting avenues yet to explore.

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Animal Vision: Starfish Can See at Last

Starfish have small compound eyes at the ends of their arms. Until recently no behavioural function had been found for them, but now it appears that starfish are able to use them to navigate to the edges of reefs from which they sometimes stray.

Michael F. Land

Although starfish are deuterostomes, and so are our very distant relatives, their compound eyes are quite unrelated to the single-chambered eyes of vertebrates, both in their construction and their cellular components (Figure 1A). Nor are they related to the much better-known compound eyes of insects and crustaceans (Figure 1B). Their function has been a mystery, since they seem to be involved in neither defence nor predation. In a recent article Garm and Nilsson [1] have been able to show that, in the starfish *Linckia laevigata*, the eyes are used to locate large landmarks, specifically the dark edges of the coral reefs that they typically inhabit. Their vision is not good, with a minimum angle of resolution of 15–30° (compared with 1° for a bee and 1′ for a human). This enables the starfish to see a 1 metre high reef from 2 metre away, but not from 4 metres away. An interesting question that this poor performance raises is why some animal groups, like the starfish, evolved eyes but never developed them to a stage where they could become of more than minimal single-task use.

There are only three animal groups in which eyes have become general-purpose sensory instruments involved in many aspects of behaviour [2]. These are the cephalopod molluscs, the arthropods, and the vertebrates. If one considers the bee as an example, the eyes are used for navigation using celestial cues and landmarks, for recognising food plants by shape and colour, for flight control using a sophisticated motion-vision system, and for recognising other members of their own or other species for mating and defence. A similar catalogue can be drawn up for most vertebrates and cephalopod molluscs.

In other animal groups vision is present, but has not been exploited to the same degree, and in many cases it is used for only a single function. For example, in the bivalve molluscs several different kinds of eye have evolved for the sole purpose of defence: Nilsson [3] describes these as 'burglar alarms'. Often these eyes are quite sophisticated: scallop eyes have unique concave mirror optics, the arc shells have small compound eyes around the mantle edge, and giant clams have pinhole eyes with modest resolution which nevertheless allow the animal to respond to a fish before it gets near enough to nibble the tentacles [4]. These all seem to be based on off-responding neurons that originally provided a shadow response: adding some optics allows the animals to respond to a predator before it is on top of them. Something similar happened in some sabellid tube-worms that have equipped themselves with compound eyes for the same defensive purpose.

'Single-purpose' eyes are not confined to use in defence: the alciopid annelids, pelagic worms that prey on other animals in the plankton, have evolved eyes with lenses that produce excellent images and would not disgrace a small fish. Similarly in the gastropod molluscs the heteropods have scanning eyes with large lenses which they again use to capture planktonic prey [5]. It is unlikely that these eyes are used for any other purposes.

Why did these eyes go nowhere beyond their one use? It is not because they were not up to the job: scallops have 2° resolution, and alciopid and heteropod eyes more like 1°, which is at least as good as most insects. It is not lifestyle either, as some of these animals are carnivores and others are