10

Mechanisms of Axon Guidance in the Developing Nervous System

Céline Plachez[*] and Linda J. Richards[†]
*Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, Maryland 21201
[†]University of Queensland, School of Biomedical Sciences and The Queensland Brain Institute, St. Lucia, Queensland 4072, Australia

Current Topics in Developmental Biology, Vol. 69

0070-2153/05 $35.00
DOI: 10.1016/S0070-2153(05)69010-2

The human brain assembles an incredible network of over a billion neurons. Understanding how these connections form during development in order for the brain to function properly is a fundamental question in biology. Much of this wiring takes place during embryonic development. Neurons are generated in the ventricular zone, migrate out, and begin to differentiate. However, neurons are often born in locations some distance from the target cells with which they will ultimately form connections. To form connections, neurons project long axons tipped with a specialized sensing device called a growth cone. The growing axons interact directly with molecules within the environment through which they grow. In order to find their targets, axonal growth cones use guidance molecules that can either attract or repel them. Understanding what these guidance cues are, where they are expressed, and how the growth cone is able to transduce their signal in a directionally specific manner is essential to understanding how the functional brain is constructed. In this chapter, we review what is known about the mechanisms involved in axonal guidance. We discuss how the growth cone is able to sense and respond to its environment and how it is guided by pioneering cells and axons. As examples, we discuss current models for the development of the spinal cord, the cerebral cortex, and the visual and olfactory systems.

I. Introduction

The establishment of correct neuronal connections is crucial for proper functioning of the nervous system. In the human brain, there are more than a billion neurons, of many different types, that assemble into highly complex neuronal networks with over 10^9 connections. To understand the brain it is essential to understand how this network of neuronal connections is achieved with such high precision as the nervous system develops. Santiago Ramón y Cajal studied the developing mammalian brain and proposed that each axon is attracted to its target cells by diffusible molecules secreted from it. Direct evidence supporting this guidance mechanism has only started to emerge during the past decade, with an increasing number of families of guidance molecules being identified. It is well established that developing axons are guided to their targets by a variety of environmental cues, including long-range diffusible and short-range surface-bound molecules that can either attract or repel the axon. The presence of these guidance cues in a temporal and spatial pattern enables the axon to navigate through the complex environment of the developing embryo to reach its correct target and to stop growing once it arrives. This chapter describes our current understanding of the mechanisms involved in the formation of neuronal connections.

II. The Growth Cone

In order to connect with targets, neurons extend processes called axons. At the tip of the extending axon is a specialized sensing device called a growth cone. In 1880, Santiago Ramón y Cajal observed and named the growth cone ("*cono de crecimiento*") as the motile structure at the leading edge of extending axons. During development of the nervous system, growth cones navigate along specific pathways, recognize their targets, and then form synaptic connections by elaborating terminal arbors. En route to their target, growth cones show stereotyped behavior by following appropriate pathways and by interacting with intermediate targets. These characteristic behaviors of axons are regulated by a combination of various types of guidance cues, including attractive and repulsive cues. How growth cones respond to guidance cues is not fixed and changes dynamically during navigation. Growth cones derive their directional information from a variety of extracellular guidance cues. These cues can take the form of both secreted and membrane-bound attractive and repulsive factors. To date, a large number of these guidance cues and their receptors have been identified (Mueller, 1999; Tessier-Lavigne and Goodman, 1996). There is also an extensive collection of actin-associated proteins that regulate the structure and dynamics of the actin cytoskeleton that makes up the motile apparatus of the growth cone (Stossel, 1993).

The growth cone has two major domains: the central domain (C domain), rich in microtubules and membranous organelles, and the peripheral domain, rich in actin filaments (Fig. 1). The peripheral domain consists of lamellipodia surrounding a central domain and filopodia extending from the outer edge of the peripheral domain. Filopodial and lamellipodial projections are sent out by the growth cone to sense its environment; they contain dense and highly polymerized actin microfilaments. Both lamellipodia and filopodia are involved in growth cone motility and undergo continuous cycles of expansion and contraction (Bray and Chapman, 1985; Goldberg and Burmeister, 1986). The cytoskeleton of the growth cone is a combination of dynamic cytoplasmic filamentous structures, including microtubules and microfilaments (Forscher and Smith, 1988; Letourneau *et al.*, 1986; Luduena and Wessells, 1973; Yamada *et al.*, 1970, 1971).

The microtubules are involved in axon extension; they extend from the neurite shaft and splay into the central part of the growth cone. Microtubules are polar structures with one end termed the "plus" end and the other end termed the "minus" end. They are composed of heterodimers of α- and β-tubulin that self-assemble to form polymers. Microtubules form dense arrays within the neurite, while they are less stable within the growth cone (Ahmad *et al.*, 1993). As microtubules enter the growth cone from the neurite shaft, the bundled microtubules separate from each other and extend

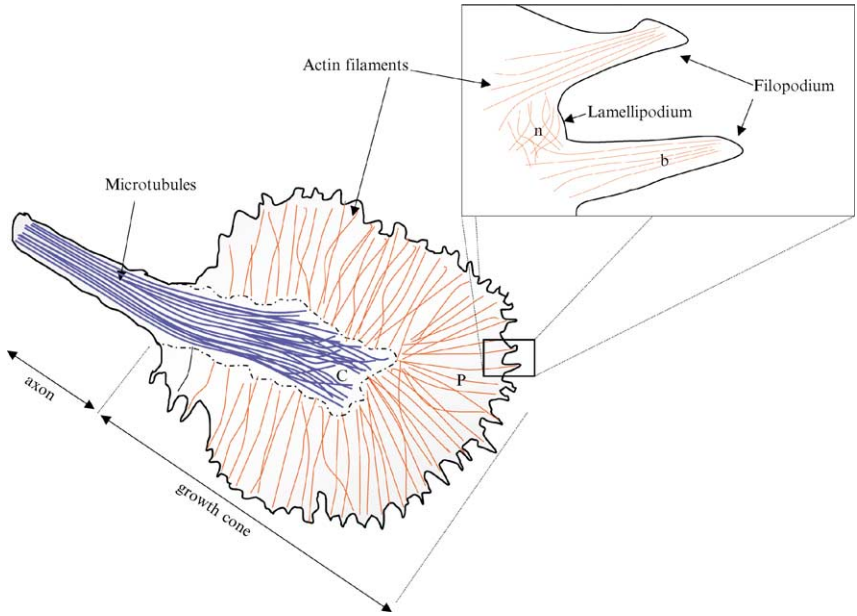


Figure 1 Schematic of the growth cone. The growing tip of the axon consists of a central domain **C** and a peripheral domain **P** with filopodium and lamellopodium. The C domain contains a dense network of microtubules, but some microtubules can also be found in the base of filipodia. Actin filaments are predominant in the P domain. Filopodium move like fingers exploring their environment. These movements are based on actin polymerization and depolymerization. Filopodial actin is organized into bundles (**b**) whereas actin filaments in lamellopodia and in the C region form an intricate network (**n**).

into the organelle-rich central domain of the growth cone in the form of individual microtubules (Tanaka and Kirschner, 1991). Growth cones also contain a large pool of soluble tubulin capable of assembling into microtubules (Letourneau and Ressler, 1984). The majority of growth cone microtubules are restricted to the C domain, but individual microtubules can also extend deep into the actin-rich peripheral domain (Gordon-Weeks, 1991; Tanaka and Kirschner, 1991).

The actin cytoskeleton is composed of two distinct microfilament subpopulations: one forms a meshwork of relatively short randomly oriented filaments in the lamellipodia, and the second is composed of parallel bundles of up to a dozen filaments that radiate from the leading edge of the lamellipodia and extend into the filopodium (Forscher and Smith, 1988; Letourneau and Ressler, 1984; Lewis and Bridgman, 1992; Luduena and Wessells, 1973; Yamada *et al.*, 1970, 1971). The actin contributes to the structure of the growth cone as well as the motility. It exits in both an

unassembled (often referred to as globular actin [G-actin] or filamentous [F-actin] form). The F-actin is polymerized from the monomeric G-actin subunits. Actin polymerization in growth cones occurs predominantly at the leading edge and is vital to growth cone motility and hence guidance.

A. Cytoskeletal Rearrangements and Growth Cone Turning

Recent studies indicate that the actin and microtubule cytoskeletons are a final common target of many signaling cascades influencing the developing neuron. Regulation of polymer dynamics and transport are crucial for proper growth cone motility. Three stages of axon outgrowth have been termed protrusion, engorgement, and consolidation (Dent and Gertler, 2003; Goldberg and Burmeister, 1986). Protrusion of the growth cone occurs by the rapid extension of filopodia and thin lamellar extensions while the plasma membrane advances forward. These extensions are primarily composed of bundled and mesh-like F-actin networks. In engorgement, the organelles and cytoplasm move forward and, finally, in consolidation, a new axon section is left behind (Bamburg, 2003; Goldberg and Burmeister, 1989). Within the cytoplasm are neurofilaments, which provide structure, and microtubules, which provide a mechanism for rapid vesicle transport (Rivas and Hatten, 1995; Schnapp *et al.*, 1986). Vesicle transport to the growth cone is vital to maintain and re-organize the membrane as it changes. Consolidation occurs when the majority of F-actin depolymerizes at the proximal part of the growth cone. These extension processes also occur during the formation of collateral branches from the main axon shaft (Dent *et al.*, 1999).

Axons are guided in new directions by the reorientation of their growth cones as well as by extension of collateral branches (O'Leary *et al.*, 1990). Axon branch formation occurs by controlled branch extension, retraction (including pruning), and stabilization. Similar to the primary growth cone, collaterals extending from the axon shaft are tipped by growth cones. In axon collateral initiation, extension and navigation require the controlled and coordinated assembly and disassembly of the neuronal cytoskeleton. At axon branch sites, the microtubules become unbundled and undergo local fragmentation within the axon shaft. This is accompanied by focal accumulation of F-actin. Similar to growth cone steering, axon branching is impaired by selective inhibition of microtubule or actin dynamics (Dent and Kalil, 2001; Rodriguez *et al.*, 2003). Cortical axon branching occurs *in vitro* through changes in growth cone morphologies and behaviors (Szebenyi *et al.*, 1998). Using a novel approach of visualizing simultaneous changes in both microtubules and actin filaments during different stages of axon branching in living cortical neurons, Dent and Kalil (2001) observed the

cytoskeletal reorganization underlying cortical axon branching. Branching from the growth cone and the axon shaft is always preceded by splaying apart of the looped or bundled microtubules, accompanied by localized accumulation of F-actin. Dynamic microtubules colocalize with F-actin in transition regions of growth cones and axon branch points, consistent with observations in fixed growth cones (Bridgman and Dailey, 1989; Challacombe *et al.*, 1996; Challacombe *et al.*, 1997; Rochlin *et al.*, 1999; Tanaka *et al.*, 1995; Williamson *et al.*, 1996), whereas F-actin is excluded from regions of stable microtubules. Recent evidence shows that several guidance molecules can also influence branching behavior (Dent *et al.*, 2004; Kornack and Giger, 2005). It has been shown that Netrin and Slit promote branching (Dent *et al.*, 2004; Wang *et al.*, 1999b) and Sema3A decreases branching (Dent *et al.*, 2004).

As the axon extends through the complex extracellular environment of the nervous system, its growth cone senses and responds to a variety of molecular guidance cues. For example, it can change from forward elongation to pauses or retraction, or it can change its direction. Growth cone turning is central to axonal navigation and is responsible for changing the direction of neurite elongation (Oakley and Tosney, 1991; Sabry *et al.*, 1991; Sretavan and Reichardt, 1993) (see Section VIII.C for pipette assay). A key event in growth cone turning may be the local realignment and advancement of microtubules (Lin *et al.*, 1994; Mitchison and Kirschner, 1988; Sabry *et al.*, 1991; Tanaka and Sabry, 1995) to establish the dominant side of a turning growth cone. When a growth cone contacts a positive guidance cue, filopodia become stabilized in the direction of neurite elongation, and microtubules are locally reoriented and advance toward the contact site (Bentley and O'Connor, 1994; Lin and Forscher, 1993; O'Connor and Bentley, 1993; Sabry *et al.*, 1991). In a similar manner, actin filaments and microtubules of growth cones may interact to accomplish turning away from a negative cue or stopping the extension of the axon altogether in a process called growth cone "collapse" (Fig. 2). Growth cone collapse involves the loss of lamellipodia and filopodia in response to a negative guidance cue.

In order to understand the mechanisms by which guidance cues direct axon growth it is important to determine how guidance cues affect F-actin dynamics and organization. Indeed, the rate of filopodial tip extension is determined by both the rate of F-actin polymerization at the filopodial tip and the retrograde displacement of polymerization filaments toward the base of the filopodium (Mallavarapu and Mitchison, 1999). The extension cycle of the lamellipodial edge is determined by both F-actin polymerization and retrograde filament transport (Lin and Forscher, 1995). Disruption of this cytoskeletal organization with drugs such as cytochalasin, nocodazole, and taxol results in alterations in growth cone morphology and motility (Forscher and Smith, 1988; Letourneau and Ressler, 1984; Letourneau *et al.*,

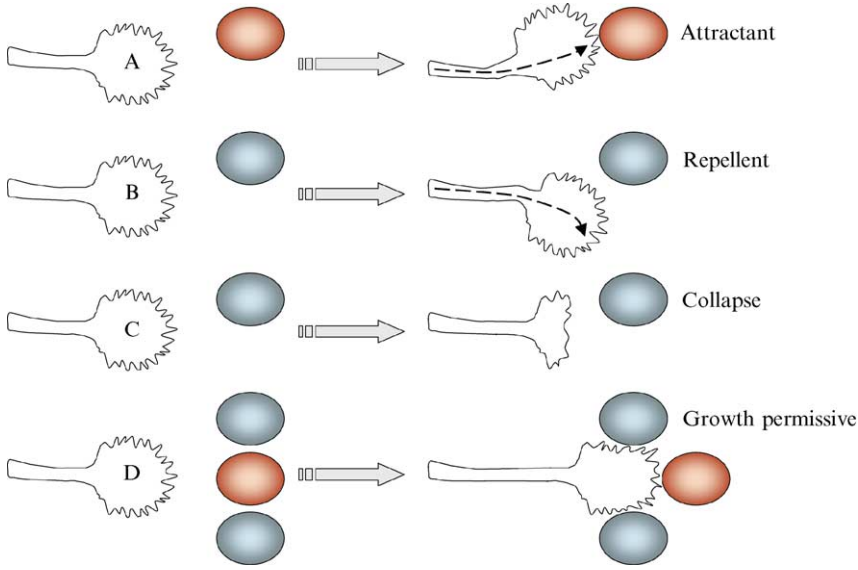


Figure 2 Growth cone behavior. The growth cone is growing toward an attractive molecule (A) or is turning away from a repulsive molecule (B). Some molecules can produce a total collapse of the growth cone (C). Some substrates are growth permissive; the growth cone grows into it with repellent cues on either side (D).

1986; Marsh and Letourneau, 1984; Neely and Gesemann, 1994; Yamada *et al.*, 1970, 1971). Disruption of either the microtubule system, by treatment with nocodozole, or the actin system, by treatment with cytochalasin, halted neuronal migration.

In vivo, growth cones are simultaneously exposed to a number of different guidance cues, both negative and positive. Repulsive guidance cues can either collapse the whole growth cone to arrest neurite outgrowth or cause asymmetric collapse leading to growth cone turning. How signals from repulsive cues are translated by growth cones into a morphological change through rearranging the cytoskeleton is unclear. Many studies examining axon repulsion have focused on the collapse response to semaphorin (Sema) 3A. Sema3A is a member of the class 3 semaphorin family that repels the axons of sensory and sympathetic neurons (Luo *et al.*, 1993). Sema3A-induced growth cone collapse is a process whereby lamellar protrusion and filopodial motility are paralyzed before the retraction of all growth cone specializations (Fan *et al.*, 1993). Fan *et al.* (1993) showed that growth cone collapse induced by Sema3A correlates with a 50% net loss of F-actin in the growth cone, suggesting that growth cone collapse could be mediated by the depolymerization of the F-actin. The morphological response to

Sema3A is similar to that caused by cytochalasin B, a plant alkaloid that inhibits actin polymerization, paralyzes normal growth cone motility, and inhibits axonal extension (Letourneau *et al.*, 1987). Collapsin response mediator proteins (CRMPs) and rac1 have been implicated in the Sema3A signaling pathway. Treatment of neurons with either anti-CRMP-62 antibodies (Goshima *et al.*, 1995) or dominant-negative rac1 (Jin and Strittmatter,1997; Kuhn *et al.*, 1999) prevents growth cone collapse by Sema3A. Stimulation of endocytosis by Sema3A also correlates with growth cone collapse. Sema3A stimulates endocytosis by the focal and coordinated rearrangement of receptor and cytoskeletal elements (Fournier *et al.*, 2000). To examine the cytoskeletal events contributing to growth cone collapse, Zhou and Cohan (2001) used three factors able to induce the collapse of extending *Helisoma* growth cones: serotonin, myosin light chain kinase inhibitor, and phorbol ester. They found that all three factors induced the collapse of extending growth cones and caused actin bundle loss in poly-L-lysine-attached growth cones without loss of actin meshwork. In addition, actin bundle loss correlated with specific filamentous actin redistribution away from the leading edge, characteristic of repulsive factors. Using time-lapse studies of extending growth cones, they showed that actin bundle loss paralleled collapse. Taken together, these results suggest that F-actin reorganization through actin bundles is the cytoskeletal mechanism underlying growth cone collapse. Growth cone collapse, or at least the initiation of collapse, may be mainly a process involving F-actin reorganization resulting in decreased actin assembly at the leading edge rather than direct inhibition of actin polymerization (Zhou and Cohan, 2001). Growth cone collapse is mediated by several signaling pathways, including the Rho-GTPases (Liu and Strittmatter, 2001) ADF (actin depolymerizing factor)/cofilin (Carlier *et al.*, 1997), LIMK (LIM kinases) (Aizawa *et al.*, 2001), and GSK-3β, FYN, and cdk5 (Eickholt *et al.*, 2002; Sasaki *et al.*, 2002).

Positive guidance cues promote and polarize protrusive activity in the direction of growth cone migration. Gundersen and Barrett (1979) were the first to observe the turning of neuronal growth cones towards sources of nerve growth factor (NGF). Since then, additional members of the neurotrophin family have been shown to act as chemoattractants (Paves and Saarma, 1997; Song *et al.*, 1997, 1998). The Arp2/3 complex nucleates the formation of new actin branches and thus initiates the step that causes forward extension of actin meshwork and subsequent extension of the membrane leading edge (Mullins *et al.*, 1998).

Ena/VASP (enabled/vasodilator-stimulated phosphoprotein) proteins play important roles in axon outgrowth and guidance. Ena/VASP activity regulates the assembly and geometry of actin networks within fibroblast lamellipodia. Ena/VASP proteins are found in the leading edge of lamellipodia (Nakagawa *et al.*, 2001) and are concentrated in the F-actin-rich region. They function

by binding to the barbed ends of filaments and competing with capping proteins, allowing for longer filament extension (Bear *et al.*, 2002). A more recent study suggests that Ena/VASP proteins play a pivotal role in the formation and elongation of filopodia along the neurite shaft and at the growth cone (Lebrand *et al.*, 2004). Netrin-1-induced filopodia formation is dependent upon Ena/VASP function and is directly correlated with Ena/VASP phosphorylation at a regulatory protein kinase A (PKA) site. Ena/VASP function is required for filopodial formation from the growth cone in response to global PKA activation. Ena/VASP proteins likely control filopodial dynamics in neurons by remodeling the actin network in response to guidance cues (Lebrand *et al.*, 2004). Krause and colleagues (2004) identified Lamellipodin as a novel Ena/VASP-binding protein. Both proteins co-localize at the tips of lamellipodia and filopodia. Lamellipodin overexpression increases lamellipodial protrusion velocity, an effect observed when Ena/VASP proteins are overexpressed or artificially targeted to the plasma membrane. Conversely, knockdown of Lamellipodin expression impairs lamellipodia formation, reduces in velocity of residual lamellipodial protrusion, and decreases in F-actin content. Lamellipodin may act as a key convergence point linking polarized phospholipid signals and small GTPases with Ena/VASP proteins to regulate the actin cytoskeleton (Krause *et al.*, 2003, 2004). A recent study also showed that RIAM, an Ena/VASP and Profilin ligand, interacts with Rap1-GTP and mediates Rap1-induced adhesion. RIAM links Rap1 to integrin activation and plays a role in regulating actin dynamics (Lafuente *et al.*, 2004). More recently, a novel protein with high similarity to the *Caenorhabditis elegans* MIG-10 protein, called PREL1 (proline-rich Ena/VASP homology domain 1 [EVH1] ligand) has been identified. PREL1 directly binds to Ena/VASP proteins and co-localizes with them at lamellipodia tips and at focal adhesions in response to Ras activation. PREL1 provides a link from Ras signaling to the actin cytoskeleton via Ena/VASP proteins (Jenzora *et al.*, 2005). The role of Ena/VASP proteins in axon guidance will be discussed further in Section IV.G.1.

Another important component of the neuronal cytoskeleton is the microtubules. Challacombe *et al.* (1996) have shown that dynamic microtubule ends are rearranged in growth cone repulsion to avoid an inhibitory guidance cue. The importance of microtubule dynamics in axonal growth and guidance has been demonstrated by the pharmacological inhibition of dynamics without affecting microtubule assembly. Low concentrations of drugs such as nocodazole, vinblastine, and taxol not only reduce axonal elongation but also prevent growth cone turning (Challacombe *et al.*, 1997; Rochlin *et al.*, 1996; Williamson *et al.*, 1996).

Studies have shown that stathmin and SCG10 are potent microtubule destabilizing factors. While stathmin is expressed in a variety of cell types and shows a cytosolic distribution, SCG10 is neuron specific and membrane

associated. SCG10 accumulates in the central domain of the growth cone, a region that also contains highly dynamic microtubules. SCG10 appears to be an important factor for the dynamic assembly and disassembly of growth cone microtubules during axonal elongation. Phosphorylation negatively regulates the microtubule destabilizing activity of SCG10 and stathmin, suggesting that these proteins may link extracellular signals to the rearrangement of the neuronal cytoskeleton (Grenningloh *et al.*, 2004).

Zhou *et al.* (2002) report that local and specific disruption of actin bundles from the growth cone peripheral domain induced repulsive growth cone turning. Meanwhile, dynamic microtubules within the peripheral domain were oriented in areas where actin bundles remained and were lost from areas where actin bundles disappeared. This resulted in directional microtubule extension, leading to axon bending and growth cone turning. In addition, this local actin bundle loss coincided with localized growth cone collapse, as well as asymmetrical lamellipodial protrusion. Regional actin bundle reorganization can steer the growth cone by coordinating actin reorganization with microtubule dynamics. This suggests that actin bundles are potential targets of signaling pathways downstream of guidance cues, providing a mechanism for coupling changes in leading edge actin with microtubules at the central domain during turning (Zhou *et al.*, 2002).

III. The Role of Pioneering Axons and Glial Guidepost Cells in Axonal Guidance

A. Pioneering Axons

Pioneering axons are the first axons to extend along a given trajectory in the brain (Fig. 3). These axons are tipped with growth cones that respond to repellents and attractants within their environment. Here we discuss the role that pioneering axons play in guiding the formation of large axonal tracts within the brain. Once the pioneer axons have established a correct path of growth, later arriving axons, called follower axons, fasciculate with the pioneers to find their target. Often a series of pioneering axons growing over short distances may be used by the main bundle to find their targets. Ablation experiments in both vertebrates and invertebrates have established the importance of pioneering axons as a mechanism for wiring the nervous system.

1. Pioneering Axons in Invertebrates

Invertebrate organisms such as flies, worms, and grasshoppers have played a prominent role in elucidating the mechanisms that are involved in axonal guidance. The advantages of these organisms include their simple nervous

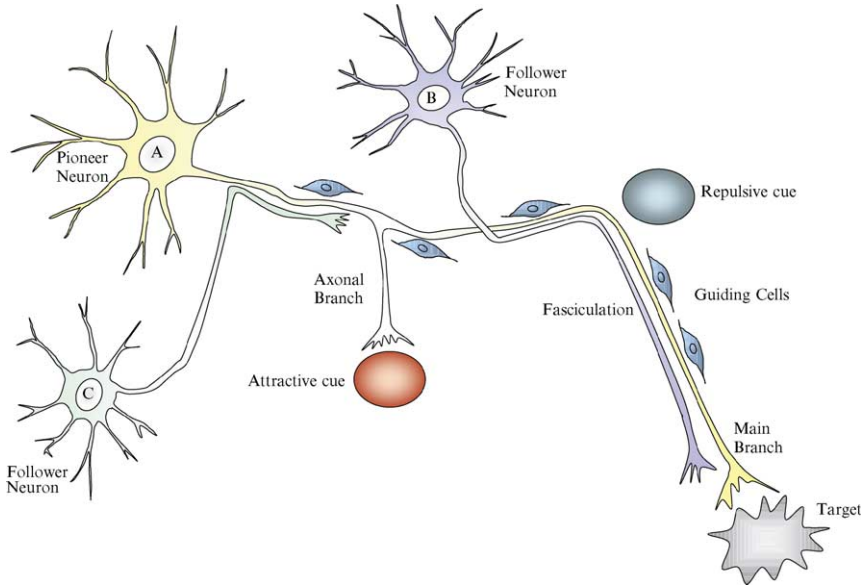


Figure 3 The pioneers. Axon of the pioneer neuron (A) grows along the pathway formed by the presence of guidepost cells as well attractive and repulsive molecules. An axonal branch can form and grow toward an attractive molecule. This axonal branch may become a stable axon, resulting in the retraction of the main branch. As pioneer neurons establish their route, follower neurons (B and C) extend their neurites. Axons of the follower neurons fasciculate with the pioneer to project to the appropriate target.

system architecture, for example, each hemisegment of the *Drosophila melanogaster* ventral nerve cord contains about 300 neurons, and the *C. elegans* nervous system has a total of 302 neurons. It is also possible to identify and follow individual neurons throughout development. Furthermore, the *Drosophila* and *C. elegans* systems allow the use of classical genetic analyses. Mutagenesis screens in both organisms have revealed many essential genes that are necessary for wiring the nervous system. In invertebrates as well as in vertebrates, axons must travel large distances to reach their final targets. Studies examining early neural development in crustacean embryos have shown the important role of pre-existing axons in guiding subsequent axon outgrowth (Whitington, 1993). Once pioneer neurons have established a route for axonal growth, axons that develop later frequently choose to selectively bundle or fasciculate with individual tracts to reach their target area.

Since axons are often pathfinding over long distances, they use guidepost cells such as pioneering axons or glial structures (also called intermediate targets) along the way to help them locate their final target. The first observation of guidepost cells came from an analysis of axon outgrowth in

the grasshopper limb bud (Bate, 1976). The Ti1 neuron navigates its way to the central nervous system (CNS) through key landmarks that are provided by neuronal somata. Ablation of these cells results in the failure of the neuron to efficiently complete its pathfinding. Guidepost cells in the limb bud of grasshopper embryos direct pioneer axons to the correct pathway (Bentley and Caudy, 1983; Keshishian and Bentley, 1983).

If particular pioneering axons are removed, the follower neurons stall and fail to extend (Bastiani *et al.*, 1986; du Lac *et al.*, 1986; Hidalgo and Brand, 1997; Raper *et al.*, 1984). Studies in grasshoppers (Bastiani *et al.*, 1984; Goodman *et al.*, 1984; Raper *et al.*, 1983a,b, 1984) demonstrate that follower axons can recognize molecules on the surface of the pioneering axon. The growth cones of two follower axons, the G and C cells, in the developing grasshopper neuropil follow four pioneering neurons (the A1, A2, P1, and P2 cells) to their targets. The G neuron extends anteriorly in the bundle and the C neuron extends posteriorly in the same bundle, but only after several other axons have joined the bundle. These results suggest that the growth cones of the G and C neurons can recognize and extend upon the four pioneering axons. This has been described as the labeled pathways hypothesis (Goodman *et al.*, 1984), which proposes that axon tracts have different molecular labels on their cell surface, labels that follower axons can specifically recognize to extend upon.

Some molecules are beginning to be identified for pioneering axon-mediated guidance. AcP neurons pioneer the anterior commissure of each grasshopper segment and extend their axons toward the midline. A cell surface glycoprotein, Lazarillo, is expressed during grasshopper embryogenesis on the surface of a subset of CNS neurons and by a group of neuroblasts, the precursors of neurons and glia (Sanchez *et al.*, 1995, 2000). When Lazarillo function was blocked, AcP neurons failed to grow into the midline; they either stopped growing or turned anteriorly. These results suggest that Lazarillo could play a role in the guidance of the AcP pioneering neurons (Sanchez *et al.*, 1995, 2000).

Goodman and Jacobs characterized neurons that pioneer the major CNS axon tracts in *Drosophila* (Jacobs and Goodman, 1989). In the embryonic *Drosophila* CNS, RP motor axons make stereotypic pathway choices involving distinct cellular contacts: (i) extension across the midline via contact with the axon and cell body of the homologous contralateral RP motoneuron, (ii) extension down the contralateral longitudinal connective through contact with connective axons and longitudinal glia, and (iii) growth into the intersegmental nerve (ISN) through contact with ISN axons and the segmental boundary glial cell (SBC). Removal of the longitudinal glia or the SBC did not adversely affect pathfinding. This suggests that the motor axons either utilized alternative axonal substrates or could still make filopodial contact with the cues in the next pathway. In contrast, RP motor axons did require contact

with the axon and soma of their contralateral RP homologue. Absence of this neuronal substrate frequently impeded RP axon outgrowth, suggesting that the next cues were beyond filopodial reach. Together, these direct ablations of putative guidepost cells in the CNS demonstrated a susceptibility by RP axons to the absence of specific cellular contacts (Whitington *et al.*, 2004).

In the nematode *C. elegans*, the gene unc-6 is required to guide pioneer axons and mesoblasts in dorsal or ventral directions of the body wall (Hedgecock *et al.*, 1990). UNC-6 is required to direct the early circumferential extension of pioneer axons in *C. elegans*, as well as the circumferential migrations of mesoblast cells (which migrate along the basement membrane surfaces facing the central pseudocoelomic cavity). Nerves form as additional axons enter the tract and fasciculate with the pioneering axon and with each other. These followers enter the tract in a set order and at stereotyped positions. Key to the role that UNC-6 plays in this process is its dynamic expression pattern (Wadsworth, 2002; Wadsworth *et al.*, 1996). The unc-129 gene, like the unc-6 gene, is required to guide pioneer motor axons along the dorsoventral axis of *C. elegans*. UNC-129 mediates expression of dorsoventral polarity information required for axon guidance and guided cell migration (Colavita *et al.*, 1998).

In *C. elegans*, synaptic guidepost cells in the vulval epithelium initiate the formation of synapses between the HSNL (hermaphrodite-specific neurons left) and HSNR (hermaphrodite-specific neurons right) and either vulval muscle cells or ventral type C (VC) neurons (Shen and Bargmann, 2003). In the absence of the guidepost cells, synaptic vesicle markers in the HSNL fail to accumulate at the normal synaptic locations, and instead form ectopic aggregates in anterior locations. *Syg-1* encodes a transmembrane immunoglobulin superfamily protein acting in the presynaptic HSN axon. Analysis of the *syg-1* mutant shows that HSNL synapses have defects similar to those observed after guidepost cell ablation, suggesting a role for SYG-1 in guidepost signaling (Shen and Bargmann, 2003). Another protein, SYG-2, binds heterophilically to SYG-1 and is expressed by guidepost cells and vulval epithelial cells. *Syg-2* mutants lack synapses at the normal location and instead form synapses onto inappropriate target cells at ectopic locations (Shen *et al.*, 2004). Molecular interactions between SYG-1 and SYG-2 on neuron and guidepost cells, respectively, contribute to synaptic specificity but also serve to suppress the formation of inappropriate synapses.

2. Pioneering Axons in Vertebrates

Pioneering axons have also been described in zebrafish. The first axons to navigate the neuroepithelium of the zebrafish brain emerge from the ventrocaudal cluster (vcc) at approximately 16 hr postfertilization (hpf) (Chitnis and Kuwada, 1990; Ross *et al.*, 1992). These axons grow caudally to pioneer

the medial longitudinal fasciculus (MLF), which is part of the larger ventral longitudinal tract (VLT) (Chitnis and Kuwada, 1990; Ross *et al.*, 1992; Wilson *et al.*, 1990), the major longitudinal tract that connects the midbrain with the hindbrain. By 18 hpf, the first axons in the forebrain emerge from the ventrorostral cluster (vrc) and grow caudally to pioneer the tract of the postoptic commissure (TPOC) (Chitnis and Kuwada, 1990; Ross *et al.*, 1992; reviewed in Hjorth and Key, 2002). Nural and Mastick (2004) also studied the postoptic commissure (POC) and characterized a system of early neurons that establish the first two major longitudinal tracts in the embryonic mouse forebrain. Each of the early axon populations first grows independently, pioneering a short segment of new tract. However, each axon population soon merges with other axons to form one of only two shared longitudinal tracts, both descending: the TPOC, and, in parallel, the stria medullaris. Thus, the forebrain longitudinal tracts are pioneered by a relay of axons, with distinct axon populations pioneering successive segments of these pathways. They identified that the transcription factor Pax6 is critical for tract formation. In Pax6 mutants, both the TPOC and the stria medullaris failed to form due to pathfinding errors of the early pioneering axons. Their results show that Pax6 could regulate longitudinal tract formation by guiding a relay of pioneer longitudinal axons in the embryonic mouse forebrain (Nural and Mastick, 2004).

Fraser and colleagues (2003) studied the POC using *in vivo* microscopy of embryonic zebrafish expressing green fluorescent protein (GFP) in the vrcs of cells in the embryonic forebrain. Their data showed that the growth of the leader pioneering axons slows down at the midline, but not the follower axons. When the leading pioneer axon is ablated, the follower axons change their midline kinetics and behave as leaders. Similarly, once the leader axons have crossed the midline they change their midline kinetics when they encounter the leading axon from the contralateral side (Bak and Fraser, 2003). These data suggest a simple model in which the level of growth cone exposure to midline cues and the presence of other axons as a substrate shape the midline kinetics of commissural axons.

In the neocortex, subplate neurons have been shown to serve as pioneering axons for thalamocortical and corticothalamic axons (De Carlos and O'Leary, 1992; Ghosh and Shatz, 1992, 1993; Ghosh *et al.*, 1990; McConnell *et al.*, 1989). When subplate neurons are ablated both corticothalamic and thalamocortical targeting are disrupted demonstrating a direct requirement for pioneering subplate neurons in forming connections between the thalamus and cortex (Ghosh and Shatz, 1992; Ghosh *et al.*, 1990; McConnell *et al.*, 1994).

In the medial cortical projection the first axons to cross the rostral cortical midline (rostral to the hippocampal commissure) are derived from neurons in the cingulate cortex (Koester and O'Leary, 1994; Ozaki and Wahlsten,

1998; Rash and Richards, 2001). These axons begin to cross the midline at embryonic day (E) 17 in the rat and E15.5 in the mouse. Neurons in the cingulate cortex project to three different regions: across the midline into the contralateral cortex, into the fornix, and ventrally into the medial septum and the diagonal band of Broca. The cingulate axons cross the midline first, followed by the neocortical axons, which grow within the tract of the cingulate pioneering axons, possibly fasciculating with the cingulate axons (Rash and Richards, 2001).

The optic chiasm also contains a subset of early generated neurons, which have been shown to be involved in retinal axon guidance (Easter *et al.*, 1993; Sretavan *et al.*, 1994, 1995). Furthermore, Cajal-Retzius cells in the hippocampus are suggested to play a role in the guidance of entorhinohippocampal axons (Del Rio *et al.*, 1997; Soriano *et al.*, 1994). In fact, two groups of pioneer neurons, Cajal-Retzius cells and GABAergic neurons, form layer-specific scaffolds that overlap with distinct hippocampal afferents at embryonic and early postnatal stages. Before the dendrites of pyramidal neurons develop, these pioneer neurons act as synaptic targets for hippocampal afferents. These findings indicate that distinct pioneer neurons are involved in the guidance and targeting of different hippocampal afferents (Super *et al.*, 1998).

Recent evidence also suggests the presence of guidepost cells in the developing mouse olfactory system. Mitral cell axons, the major efferents of the olfactory bulb, caudally elongate in a very narrow part of the lateral telencephalon and make a stereotyped turn toward the amygdala (Brunjes and Frazier, 1986; Schwob and Price, 1984; Shipley *et al.*, 1995). The axons collectively form a fiber bundle called the lateral olfactory tract (LOT). Organotypic co-culture of olfactory bulb with various regions of the mouse telencephalon showed that mitral cell axons are guided by biochemical cues that are strictly localized to the telencephalon (Sugisaki *et al.*, 1996). Indeed, intrinsic cells in the telencephalon play a directional role in the guidance of mitral cell axons (Sugisaki *et al.*, 1996). Mitral cell axons selectively grew along the LOT cells *in vivo* and in co-culture. Ablation of LOT cells in organotypic cultures caused mitral cell axons to stall. These results suggest that LOT cells function as guidepost cells for mitral cell axons (Sato *et al.*, 1998).

B. Intermediate Targets and Glial Guidepost Cells

Often, the final target of an axon is a long distance away. In order to navigate toward their final target, axons use intermediate targets to guide them along the correct path of growth. Such intermediate targets can be pioneering axon populations such as those described above or glial cells or structures present along the pathway that secrete guidance factors (Fig. 3).

Once a growth cone encounters such an intermediate target, it slows and transforms its morphology, apparently looking for further molecular "directions." Guidepost cells have been identified in *Drosophila* (Auld, 1999; Hidalgo, 2003) in which glia secrete guidance cues and express cellular cues on their surface that guide axonal outgrowth. Glia act as intermediate targets in growth cone guidance. Because guidepost cells were found in invertebrates, similar guiding mechanisms have been postulated in the mammalian CNS. In vertebrates, primitive glial cells are involved in guiding pioneering growth cones in the developing spinal cord (Kuwada, 1986; Singer *et al.*, 1979), the ventral roots (Nordlander *et al.*, 1981), the optic nerve (Silver and Sapiro, 1981; Silver and Sidman, 1980), the auditory system (Carney and Silver, 1983) and the developing corpus callosum (Silver *et al.*, 1982, 1993). During embryonic development, glia cells are required for the formation of the CNS (Fitch and Silver, 1997). They also define boundaries between different brain areas or between functional subdomains within the same area (Cooper and Steindler, 1986; Garcia-Abreu *et al.*, 1995; Mastick and Easter, 1996; Silver, 1994; Silver *et al.*, 1993; Yoshida and Colman, 2000). These glial boundaries serve to prevent axons from straying from their correct path of growth (Fitch and Silver, 1997). At the *Drosophila* midline, glia function as guidepost cells for commissural and ipsilaterally projecting axons to determine which axons cross the midline and which do not (Hidalgo and Booth, 2000; Jacobs and Goodman, 1989; Kidd *et al.*, 1999). To better understand the origin of commissures, a mutant screen was carried out for flies with defective commissure formation. Commissureless (Comm) is one of the mutations isolated. In Comm mutants, commissural growth cones initially orient toward the midline, but none actually cross it. Rather, any short medially oriented processes are retracted, and the axons remain exclusively on their own side, producing the commissureless axon guidance phenotype (Seeger *et al.*, 1993). Robo was isolated in the same screen, and leads to the opposite misrouting; some growth cones that normally extend only on their own side project across the midline in Robo mutants. The phenotype of these two genes suggests that they encode components of attractive and repulsive signaling systems at the midline. Comm is able to downregulate levels of the Robo protein on the cell surface, which is necessary for axons to cross the midline. Comm is expressed and required in both commissural neurons and midline cells for correct midline crossing (Georgiou and Tear, 2002). It is suggested that the presence of Comm in the commissural neurons may encourage midline crossing. Comm protein accumulates at the axon surface within the commissural region, using a mechanism that is likely to involve Comm expressed by midline glia (Couch and Condron, 2002; Georgiou and Tear, 2002; Keleman *et al.*, 2002; Keleman *et al.*, 2005; Myat *et al.*, 2002).

Three pairs of midline glia, as well as the medial precursor 1 (MP1) and ventral unpaired median (VUM) neurons, are present at the midline of the *Drosophila* ventral nerve cord (Klambt *et al.*, 1991). Analysis of mutants defective in midline cell development reveal essential roles for these cells in the formation of the *Drosophila* ventral nerve cord. In the single-minded (*sim*) mutant, midline cells fail to differentiate and ultimately die. Consequently, commissures do not form and the longitudinal connectives collapse into a single fused tract at the midline (Klambt *et al.*, 1991; Thomas *et al.*, 1988).

Glial cells may also function to sort ipsilaterally from contralaterally projecting axons. At the optic chiasm, retinal ganglion cell (RGC) axons contact glial cells known as the glial palisade. These glia may contribute to retinal axonal divergence at the chiasm (Erskine *et al.*, 2000; Marcus *et al.*, 1995). Glial populations are also associated with the formation of commissures such as the anterior commissure (Cummings *et al.*, 1997; Pires-Neto *et al.*, 1998), the corpus callosum (Shu and Richards, 2001; Silver *et al.*, 1993) (see Section V.B), and decussating axons in the hindbrain, brain stem, and the corticospinal tract (Joosten *et al.*, 1989; Mori *et al.*, 1990; Van Hartesveldt *et al.*, 1986). Glial cells in the floor plate of the spinal cord guide commissural axons of the dorsal spinal cord (Altman and Bayer, 1984; Tessier-Lavigne *et al.*, 1988a).

IV. Molecules Involved in Axonal Guidance

Neuronal growth cones are guided to their targets by both short- and long-range cues. Both attractive and repulsive cues are equally important for the guidance of growth cones to their appropriate targets. Most axon guidance molecules identified were discovered first in *Drosophila* or *C. elegans*. In some cases, guidance factors were simultaneously directly purified from vertebrate systems through massive purification efforts. There are four major families of classic axon guidance molecules: Netrins, Slits, semaphorins, and ephrins and their receptors. However, a number of other molecules have been shown to guide axons, including morphogens, steroids, and extracellular matrix and adhesion molecules. Here we briefly review these molecules and some of the intracellular signaling mechanisms they use.

A. Netrins and DCC

Netrin-1, whose name stems from the Sanskrit term "one who guides," was originally purified in vertebrates as a floorplate-derived chemoattractant by an exhaustive purification from over 10,000 chick brains that can promote commissural axon outgrowth (Kennedy *et al.*, 1994; Serafini *et al.*, 1994). Netrins are secreted proteins that act on neural cells through transmembrane

receptors of the Neogenin A2b receptor, DCC (deleted in colorectal carcinomas), and UNC5H families (Mehlen and Mazelin, 2003) (Fig. 4). Netrins make up a small family of secreted, laminin-related molecules with multifunctional roles in axon guidance, acting as context-dependent chemoattractants or chemorepellents. Several members of the Netrin family have been identified in a variety of species: the UNC-6 gene product in nematodes;

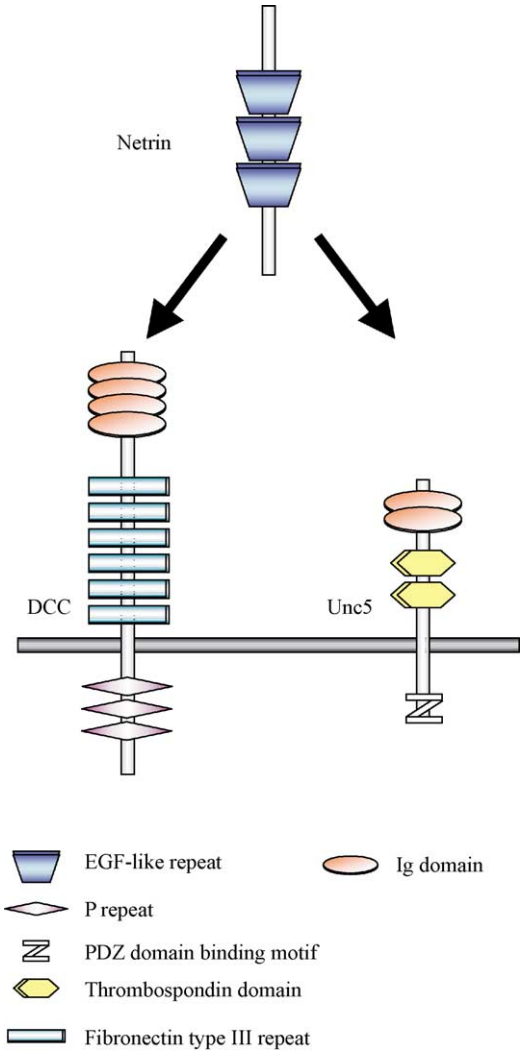


Figure 4 Netrins and DCC.

netrin-A and netrin-B in *Drosophila;* netrin-1 and netrin-2 in chicks; netrin-1, netrin-3, netrin-G1, netrin-G2, and netrin-4/β-netrin in mice; and the NTN2L (netrin-2-like) in humans (Koch *et al.*, 2000; Nakashiba *et al.*, 2002; Serafini *et al.*, 1994, 1996; Wang *et al.*, 1999a). The Netrins encode ~60–80 kDa secreted proteins that share homologous domains with laminin (Banyai and Patthy, 1999; Serafini *et al.*, 1994). The *C. elegans* netrin (UNC6) was the first member of this family identified by examining mutants with uncoordinated (unc) phenotypes (Hedgecock *et al.*, 1990; Ishii *et al.*, 1992). Netrins regulate the development of commissural axons in both the spinal cord and brain, including the corpus callosum, hippocampal commissure, and the optic chiasm (Serafini *et al.*, 1996). Netrins are not only involved in axon guidance but also play central roles in the migration of neurons, glial oligodendrocyte precursors, and mesodermal cells during embryogenesis (Alcantara *et al.*, 2000; Bloch-Gallego *et al.*, 1999; Hamasaki *et al.*, 2001; Lim and Wadsworth, 2002; Spassky *et al.*, 2002; Su *et al.*, 2000; Sugimoto *et al.*, 2001; Tsai *et al.*, 2003; Yee *et al.*, 1999). More recently, it has been shown that the Netrin-1 receptor DCC is phosphorylated by Fyn and that phosphorylation is required for DCC function. This suggests that Fyn is essential to initiate the responses of axons to Netrin-1 (Meriane *et al.*, 2004).

B. Semaphorins, Neuropilins, and Plexins

Semaphorins (Semas) were originally identified in invertebrates (Kolodkin *et al.*, 1992). Sema3A, the prototype vertebrate member of the semaphorin family, was initially purified from chick brain extracts on the basis of its collapse-inducing activity on cultured dorsal root ganglion (DRG) growth cones (Luo *et al.*, 1993). The semaphorin family contains both secreted and membrane-bound members, divided into eight classes (Semaphorin Nomenclature Committee, 1999). The first two classes represent invertebrate semaphorins, classes 3–7 represent vertebrate semaphorins, and the eighth class comprises viral semaphorins. Of the vertebrate classes (Fig. 5), class 3 contains secreted semaphorins and classes 4–7 contain transmembrane or membrane-anchored semaphorins. All semaphorins share a conserved, 500-amino-acid motif, termed the sema domain (Kolodkin *et al.*, 1993; Luo *et al.*, 1993). The first semaphorin receptor identified was neuropilin (Chen *et al.*, 1997; Giger *et al.*, 1998; He and Tessier-Lavigne, 1997; Kolodkin *et al.*, 1997). The two main protein families now known to be involved in mediating semaphorin responses are the neuropilins (two members, Npn1 and Npn2; Fig. 5) and plexins (nine members), Plexin A1–4, Plexin B1–3, Plexin C1, and Plexin D1 (de Wit and Verhaagen, 2003; Fujisawa and Kitsukawa, 1998; Pasterkamp and Kolodkin, 2003; Raper, 2000; Tamagnone *et al.*, 1999; Winberg *et al.*, 1998). Invertebrate semaphorins, membrane-associated

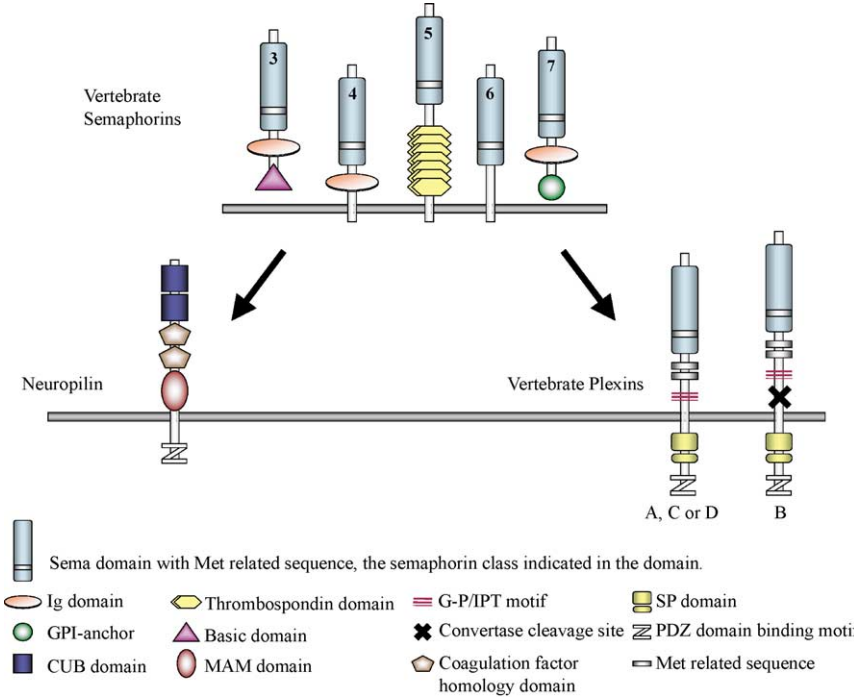


Figure 5 Semaphorins, Neuropilins, and Plexins.

semaphorins in vertebrates, and viral semaphorins have been shown to interact directly with plexins (Comeau *et al.*, 1998; Tamagnone *et al.*, 1999; Winberg *et al.*, 1998). Vertebrate class 3 secreted semaphorins, however, utilize neuropilin proteins as ligand-binding obligate co-receptors, which assemble a semaphorin/neuropilin/plexin signaling complex (Chen *et al.*, 1997; He and Tessier-Lavigne, 1997; Takahashi *et al.*, 1999; Tamagnone *et al.*, 1999).

All four members of the plexin-A subfamily can function as a signal-transducing component in class 3 semaphorin receptor complexes. Plexin A1, Plexin A2, and Plexin A4, when expressed in a complex with Npn-1 or Npn-2, induce a contraction of the cytoskeleton of COS cells in response to class 3 semaphorins (Suto *et al.*, 2003; Takahashi and Strittmatter, 2001). Cultured sympathetic axons derived from Plexin A3 knockout mice, however, are no longer repelled by Sema3F and partially lose their responsiveness to Sema3A (Cheng *et al.*, 2001). The response to class 3 semaphorins is determined by the complement of neuropilins on the cell surface, and

additional specificity is achieved by the combination of plexin-As expressed in the neuron.

Npn1 can bind another ligand, vascular endothelial growth factor (VEGF) (Soker *et al.*, 1998), and several proteins other than neuropilins and plexins can participate in semaphorin receptor complexes. These include the cell adhesion molecule L1, which transduces a chemorepulsive response to Sema3A in cortical neurons, together with Npn1 (Castellani *et al.*, 2000, 2002). To study the role of Npn1-Sema signaling independent of VEGF/Npn1 signaling, Gu and colleagues specifically mutated the Sema-binding domain of Npn1, while leaving the VEGF-binding domain intact (Gu *et al.*, 2003). These mice have axonal guidance defects in the formation of the entorhino-hippocampal pathway, cranial and spinal nerves, sensory projections to the inner ear, and the corpus callosum (Gu *et al.*, 2003).

Recently, integrins have been shown to function as receptors for Sema7A, which induces olfactory axon growth without the need for plexins (Pasterkamp *et al.*, 2003). Finally, recent data suggest that the attractive/repulsive signaling of Sema5A can be modulated by interactions with the extracellular matrix (Kantor *et al.*, 2004; see further below).

C. Slits and Robos

Slit proteins make up a family of multifunctional guidance cues with putative roles in regulating neuronal migration (Wu *et al.*, 1999), axonal and dendritic branching (Ozdinler and Erzurumlu, 2002; Wang *et al.*, 1999b; Whitford *et al.*, 2002), and axon guidance (Brose *et al.*, 1999; Kidd *et al.*, 1999). These large glycoproteins are conserved across species with three family members (Slit1, Slit2, and Slit3) identified in the developing and adult mammalian nervous systems (Brose *et al.*, 1999; Marillat *et al.*, 2002). They contain several protein motifs: leucine-rich repeats, EFG repeats, and a laminin G domain (Rothberg *et al.*, 1990) (Fig. 6). The three vertebrate Slits have overlapping, but distinct, patterns of expression throughout development and in adulthood (Holmes *et al.*, 1998; Itoh *et al.*, 1998; Marillat *et al.*, 2002; Piper *et al.*, 2000; Yuan *et al.*, 1999). Knockout studies demonstrate that Slits 1 and 2 play critical roles in the formation of several mammalian fiber tracts, including corticofugal, thalamocortical, callosal, optic, and the lateral olfactory tract (Bagri *et al.*, 2002; Keleman *et al.*, 2002; Nguyen-Ba-Charvet *et al.*, 2002; Plump *et al.*, 2002). The Robo family of transmembrane proteins are the receptors for Slits (Brose *et al.*, 1999; Fricke *et al.*, 2001; Kidd *et al.*, 1998; Zallen *et al.*, 1998). Three mammalian homologues of *Drosophila* Robo have been identified (Robo1, Robo2, and Rig-1). Robos are members of the immunoglobulin (Ig) superfamily; their ectodomain contains five Ig-like repeats followed by three fibronectin type III

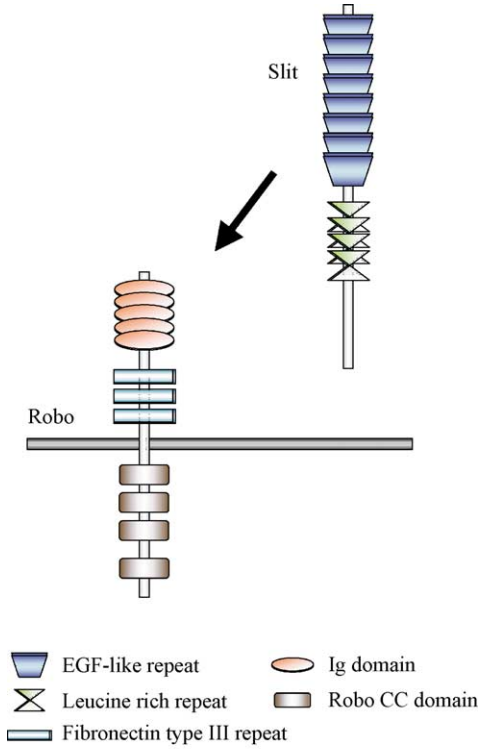


Figure 6 Slits and Robos.

repeats (Kidd *et al.*, 1998). In *Drosophila*, ipsilaterally projecting axons and decussated commissural axons expressing Robo are prevented from inappropriate crossing/re-crossing of the midline via interaction with Slit (Kidd *et al.*, 1999). Genetic mutations in either Robo or Slit lead to aberrant crossing and re-crossing, or failure of these axons to leave the midline (Kidd *et al.*, 1999). In *Drosophila*, commissural axons acquire a postcrossing sensitivity to Slit resulting from increased surface expression of Robo. This occurs via a mechanism that includes inactivation of Comm, an intracellular sorting protein that normally targets Robo for endosomal degradation (Keleman *et al.*, 2002). In Comm mutants, commissural growth cones initially orient toward the midline, but none actually cross. Rather, any short medially oriented processes are retracted, and the axons remain exclusively on their own side, producing the commissureless phenotype (Seeger *et al.*, 1993). Downregulation of Robo is necessary for axons to cross the midline. Comm is expressed and required in both commissural neurons and midline

cells for correct midline crossing (Georgiou and Tear, 2002). It is suggested that the presence of Comm in commissural neurons may encourage midline crossing. Comm protein accumulates at the axon surface within the commissural region, using a mechanism that is likely to involve Comm in the midline glia. However, Comm activity does not extend beyond the midline, allowing Robo levels to increase at the growth cone surface and initiate sensitivity to the midline inhibitor Slit that encourages axon growth away from the midline and prevents re-crossing (Couch and Condron, 2002; Georgiou and Tear, 2002). Thus, Comm controls axon guidance at the midline by regulating surface levels of Robo. Two different models have been proposed to explain how Comm regulates Robo. The first model proposes that Comm controls the sorting of Robo at the trans-Golgi network (Keleman *et al.*, 2002). The second model proposes that Comm controls Robo by acting at the plasma membrane. In this model, Comm does not block the delivery of Robo to the growth cone but instead rapidly removes it by endocytosis (Myat *et al.*, 2002). In a genetic rescue assay for Comm, Dickson and colleagues showed that midline crossing does not require the presence of Comm in midline cells (as proposed in the second model). They also showed by monitoring the trafficking of Robo that Comm prevents the delivery of Robo to the growth cone (as predicted in the first model) (Keleman *et al.*, 2005).

Slit proteins are also alternatively spliced in both mouse and human, implying that multiple Slit protein isoforms may exist (Little *et al.*, 2002). Recent studies of the Slit1 protein show an alternatively spliced mRNA product for *slit1* found specifically in the vertebrate nervous system (Tanno *et al.*, 2004). This variant was designated *slit1α*. *Slit1α* is specifically expressed in rat brain, but not heart or kidney, suggesting that *slit1α* plays a role in nervous system development. *Slit1α* expression was found in the cerebral cortex and the hippocampus. *In vivo*, *slit1α* acts as a chemorepellent of olfactory bulbs axons (Tanno *et al.*, 2004).

D. Ephrins and Eph

Ephrins and their tyrosine kinase receptors, the Eph molecules, are divided into two classes: ephrin-As, which are anchored to the membrane via glycosyl-phosphatidytinositol (GPI) linkage, and ephrin-Bs, which are transmembrane proteins (Fig. 7). The ephrin-A subclass contains ephrins A1 to A5 and the ephrin-B subclass has three members, ephrins B1 to B3 (Kullander and Klein, 2002). Eph receptors are divided into an A subclass that contains eight members (EphA1–EphA8), and a B subclass that contains five members (EphB1–EphB4, EphB6) (Cutforth and Harrison, 2002; Huot, 2004; Wilkinson, 2001). A-type receptors bind to most or all A-type ligands, and

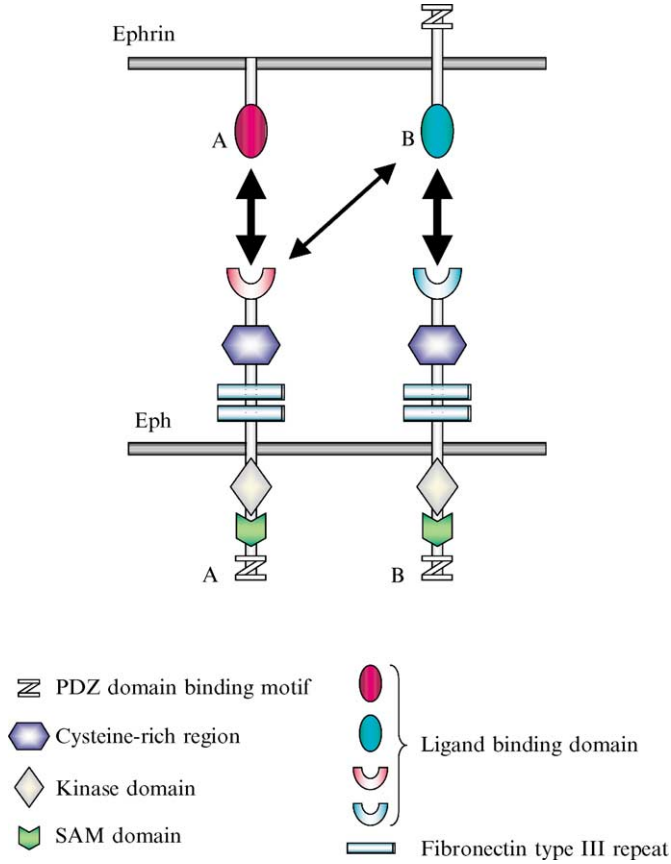


Figure 7 Ephrins and Eph.

B-type receptors bind to most or all B-type ligands. The primary exception is the EphA4 receptor, which has been shown to interact with members of both class A and class B ephrins. The extracellular domain of the Eph receptors contains the ligand-binding domain, a cysteine-rich region, and two fibronectin type III repeats. The cytoplasmic domain of the Eph receptors can be divided into four functional units: the juxtamembrane domain that contains two conserved tyrosine residues, a classical protein tyrosine kinase domain, a sterile-α-motif, and a PDZ domain binding motif (Huot, 2004; Kullander and Klein, 2002). Since both the ephrin ligands and the Eph receptors are membrane bound, the interactions between ephrins and the Eph receptors require intracellular contact. An Eph receptor can also act as a ligand in the same way that an ephrin ligand can act as a receptor (Mellitzer *et al.*, 1999).

Ligand binding induces forward signaling, but ephrins can also signal via the receptor, which is called reverse signaling (Bruckner *et al.*, 1997; Holland *et al.*, 1996).

The ephrin–Eph receptor system regulates many cellular functions that depend upon cytoskeletal remodeling, such as axon guidance and synaptic plasticity. The influence of Eph–ephrin activation on cell behavior differs depending on the cell type. They are generally associated with repulsion of neighboring cells or of cellular processes, such as the neuronal growth cone. However, in some cases, Eph–ephrin activation leads to increased adhesion or attraction. Both classes have been implicated as regulators of axon guidance. Eph/ephrin molecules have also been implicated in guiding commissural axons in the spinal cord and brain (Henkemeyer *et al.*, 1996; Hu *et al.*, 2003; Imondi and Kaprielian, 2001; Imondi *et al.*, 2000; Orioli *et al.*, 1996; Palmer and Klein, 2003; Yokoyama *et al.*, 2001). Ephrin–Eph complexes also regulate axon guidance in the visual system. In particular, ephrin-A ligands and Eph A receptors mediate repulsion that is typically involved in regulating the mapping of retinal axons along the anterior-posterior axis (reviewed in Huot, 2004; see also Section VIII.B for the stripe assay). Topographic mapping of the anterior-posterior tectal/superior collicular axis is dependent upon expression patterns of EphA/ephrin-A (Brown *et al.*, 2000; Feldheim *et al.*, 2000), while EphB/ephrin-B signaling is important for mapping along the dorsoventral axis (Hindges *et al.*, 2002; Mann *et al.*, 2002b).

E. Morphogens

Morphogens are known for their effects on cell fate determination during development. Local concentration gradients convey positional information used during organization of the major body axes, limb development, and patterning of the nervous system. Recent studies suggest that in addition to these roles, morphogens, including members of the Wnt family, bone morphogenetic protein (BMP) family, and Sonic Hedgehog (Shh), might also function in axon guidance. For instance, a knockout of the Wnt receptor, Frizzled-3, results in severe defects in several major fiber tracts in the vertebrate forebrain (Wang *et al.*, 2002c), and Wnt-3 slows axon outgrowth and may mediate terminal branching of vertebrate muscle afferents (Krylova *et al.*, 2002). Wnt proteins are a large family of diffusible factors (7 members in *Drosophila* and 19 in humans) that play several important roles in both embryonic development and adult function. Wnt proteins have well-established roles in early cell fate decisions and embryonic patterning (Wodarz and Nusse, 1998), but they have also been implicated in synaptic remodeling and terminal arborization within the developing CNS (Hall *et al.*, 2000;

Krylova *et al.*, 2002; Packard *et al.*, 2002) and in planar cell polarity (Bhanot *et al.*, 1996; Vinson and Adler, 1987). In the *Drosophila* ventral nerve cord, Wnt-5 binding to the receptor tyrosine kinase Derailed is required for targeting axons to the appropriate midline commissure (Yoshikawa *et al.*, 2003). Genetic and biochemical data indicate that Wnt5 binds Derailed and prevents Derailed-expressing axons from entering the posterior commissure (Garrity, 2003). The dorsal spinal cord commissural neurons form several ascending somatosensory pathways. During embryonic development, they project axons to the ventral midline. At the floor plate, commissural axons cross the midline, enter the contralateral side of the spinal cord, and make a sharp anterior turn toward the brain (Bovolenta and Dodd, 1990). *In situ* hybridizations of Wnts in developing mouse embryos revealed that Wnt4, Wnt7b, and Wnt5a are expressed in areas where postcrossing axons turn anteriorly. Wnt4 was found specifically enriched in the floor plate and the ventricular zone, exhibiting a decreasing anterior-to-posterior gradient along the entire length of the floor plate (Lyuksyutova *et al.*, 2003). A similar Wnt4b gradient in the floor plate was found in zebrafish embryos at similar stages (Liu *et al.*, 2000). A directed source of Wnt4 protein attracted postcrossing commissural axons (Lyuksyutova *et al.*, 2003). Commissural axons in mice lacking the Wnt receptor Frizzled3 displayed anterior-posterior guidance defects after midline crossing. Thus, Wnt-Frizzled signaling guides commissural axons along the anterior-posterior axis of the spinal cord (Lyuksyutova *et al.*, 2003).

A role for BMPs in axonal guidance in vertebrates has emerged from studies of commissural axon trajectories in the developing spinal cord. Many commissural neurons differentiate adjacent to the roof plate at the dorsal midline of the spinal cord and extend axons ventrally (Dodd *et al.*, 1988; Holley, 1982). Signals derived from the floor plate contribute to the ventral trajectory of commissural axons (Colamarino and Tessier-Lavigne, 1995). The initiation of ventral growth of commissural axons may be mediated by a chemorepellent signal emanating from the roof plate. *In vitro* studies have shown that the roof plate is the source of a diffusible repellent activity that orients commissural axons in explants and that this repellent activity can be blocked by antagonists of BMPs (Augsburger *et al.*, 1999). At the time that commissural axon extension is initiated, *Bmp6*, *Bmp7*, and *Gdf7* are expressed in the rodent roof plate (Augsburger *et al.*, 1999; Lee *et al.*, 1998). BMP7 can mimic the repellent activity of the roof plate on commissural axons in explants *in vitro*, whereas BMP6 has only a low level of repellent activity, and GDF7 is inactive. Moreover, BMP7 elicits commissural growth cone collapse, illustrating the direct nature of its action. Roof plate tissue isolated from *Bmp7* mutant mice exhibits a marked reduction in roof plate-repellent activity *in vitro* (Augsburger *et al.*, 1999). Together, these findings suggest that BMPs can act as axon guidance signals that contribute to the

chemorepellent activity of the roof plate. A more recent study analyzing roof plate-repellent activity in mice lacking Bmp7, Bmp6, and *Gdf7* alone and in pair-wise combinations show that both Gdf7 and Bmp7 but not Bmp6 are required for the ability of the roof plate to orient commissural axons. GDF7 and BMP7 heterodimerize, and the heterodimer is a more potent repellent than the BMP7 homodimer for commissural axons These results suggest that the GDF7:BMP7 heterodimer functions as a roof plate-derived repellent that establishes the initial ventral trajectory of commissural axons (Butler and Dodd, 2003).

Other evidence for morphogen involvement in axon guidance includes defects in retinal ganglion cell projections in mice deficient in the BMP receptor, BMPR-IB (Liu *et al.*, 2003). Shh can inhibit the outgrowth of neurons from retinal explants *in vitro* (Trousse *et al.*, 2001). Shh is a secreted protein that interacts with two transmembrane proteins, Patched (ptc) and Smoothened (smo). Ptc binds to shh, whereas smo is involved in signal transduction. In the absence of shh, ptc inhibits smo. A recent study by Charron and colleagues (2003) shows that Shh from midline structures collaborates with netrin-1 to guide commissural axons. This new role for the morphogen Shh raises the possibility that principles similar to those used to establish positional information in embryonic patterning are also employed during axon navigation (reviewed in Salinas, 2003).

F. Steroids

Steroid hormones may also induce directed neurite outgrowth. Estrogen elicits a significant enhancement of neurite outgrowth and differentiation within organotypic explant cultures of hypothalamus, preoptic area, and cerebral cortex (Toran-Allerand, 1976, 1980, 1984). Forebrain neurons coexpress nerve growth factor (NGF) receptors and estrogen receptor mRNA (Miranda *et al.*, 1993; Toran-Allerand *et al.*, 1992), and NGF significantly increases nuclear estrogen binding in cortical but not basal forebrain explants (Miranda *et al.*, 1996). Steroid/neurotrophin interactions may stimulate the synthesis of proteins required for neuronal differentiation, survival, and maintenance of function (Toran-Allerand, 1996). Estrogen and neurotrophin may regulate the same broad array of cytoskeletal and growth-associated genes involved in neurite growth and differentiation (Singh *et al.*, 1999).

The estrogen receptor, a nuclear transcription factor, is widely expressed in the developing forebrain (Toran-Allerand, 2004). Two mammalian estrogen receptors have been described, ER-α (White *et al.*, 1987), mediating most of the transcriptional action of estrogen in the brain, and ER-β (Kuiper *et al.*, 1996; Tremblay *et al.*, 1997). Mice lacking ER-β exhibit a reduction in the expression of genes involved in neuronal migration and axonal guidance,

such as semaphorin G, syndecan 3, and reelin. Therefore, it appears that ER-β influences migration of neurons during development and neuronal survival throughout life (Wang *et al.*, 2002a, 2003).

The principal nucleus of the bed nuclei of the stria terminalis (BSTp) pathway to the anteroventral periventricular nucleus of the preoptic region (AVPV) develops in a sexually dimorphic pattern, suggesting a directed mechanism of axonal guidance (Hutton *et al.*, 1998). *In vitro*, addition of testosterone to BSTp to AVPV co-cultures induces neurite extension (Ibanez *et al.*, 2001). Testosterone induces a target-derived, diffusible chemotropic activity that results in a sexually dimorphic pattern of connectivity (reviewed in Simerly, 2002). The AVPV projects to the gonadotropin-releasing hormone (GnRH) neurons and the tuberoinfundibular dopaminergic (TIDA) neurons. Expression of ER-α by GnRH neurons (Skynner *et al.*, 1999) could suggest that estrogen may also direct the development of projections from the anteroventral periventricular nucleus to the GnRH neurons (Simerly, 2002).

The role of estrogen in branching has also been reported in invertebrates. Ecdysteroids, the insect steroids that trigger metamorphosis, control both regression and outgrowth *in vivo* and stimulate neuritic growth in cultured pupal leg motor neurons. Ecdysteroid enhances neuritic branching by altering growth cone structure and function, suggesting that hormonal modulation of cytoskeletal interactions contributes significantly to neuritic remodeling during metamorphosis (Matheson and Levine, 1999).

G. Intracellular Signaling Mechanisms

1. Ena/VASP

Ena/VASP proteins are a conserved family of actin regulatory proteins made up of Ena/VASP homology domain 1 (EVH1) and EVH2 domains and a proline-rich central region (Fig. 8). Mammalian Ena/VASP members are Mena, n-Mena, Ena/VASP like (EVL), and VASP. The members are 60–70% identical to each other. The Ena/VASP family is involved in Abl and/or cyclic nucleotide-dependent protein kinase signaling pathways. They have been implicated in actin-based processes such as fibroblast migration, axon guidance, and T-cell polarization and are important for the actin-based motility of the intracellular pathogen *Listeria monocytogenes* (Chakraborty *et al.*, 1995; Gerstel *et al.*, 1996; Gertler *et al.*, 1996; Pistor *et al.*, 1995; Smith *et al.*, 1996). Vertebrate Ena/VASP proteins are substrates for PKA/PKG serine/threonine kinases. Phosphorylation by these kinases appears to modulate Ena/VASP function within cells, although the mechanism underlying this regulation remains to be determined. Ena/VASP are also crucial factors in regulating actin dynamics and associated processes such as cell–cell adhesion.

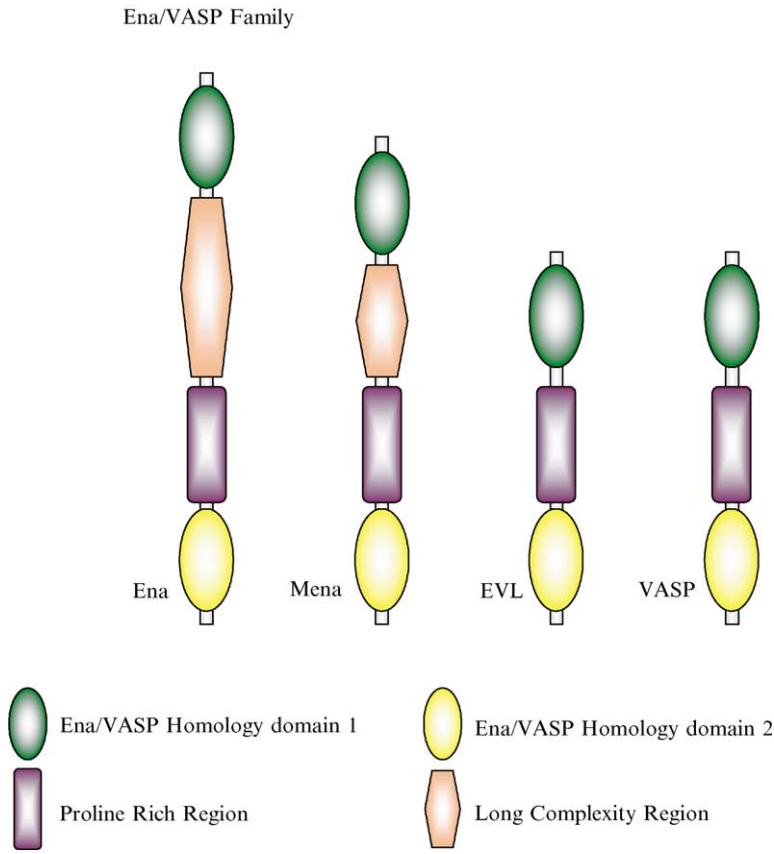


Figure 8 Ena/VASP.

Evidence also suggests that these have inhibitory functions in integrin regulation, cell motility, and axon guidance (Krause *et al.*, 2003).

Mena and Mena/VASP mouse mutants have defects in several major axonal tracts (Lanier *et al.*, 1999), including the corpus callosum, the hippocampal commissure, the anterior commissure, and the pontocerebellar pathway. Ena/VASP proteins are highly expressed in the developing cortical plate in cells bordering reelin-expressing Cajal-Retzius cells and in the intermediate zone. Inhibition of Ena/VASP function through retroviral injections *in utero* leads to the aberrant migration of early-born pyramidal neurons in the superficial layers of both the embryonic and the postnatal cortex in a cell-autonomous fashion. The results demonstrate that Ena/VASP proteins play a key role in regulating neuronal migration and layering within the developing mouse neocortex (Goh *et al.*, 2002).

2. Rho GTPases

Rac, Rho, and Cdc42 are small GTPases of the Rho family. They have been shown to regulate actin organization in non-neuronal cells (Hall, 1998), as well as cytoskeletal dynamics in neuronal growth cones (Luo *et al.*, 1997). A model has been proposed (Hall, 1998; Mueller, 1999) in which attractive guidance cues activate Rac and Cdc42 to promote growth cone advance, whereas repulsive guidance cues activate Rho to inhibit growth and induce retraction (Dickson, 2001). For example, ephrin-A5 activates Rho and inhibits Rac in cultures of retinal ganglion cells. Both Rho and its downstream effector Rho kinase are required for growth cone collapse (Wahl *et al.*, 2000). Rac proteins mediate axon guidance, outgrowth, and branching as well as suppress the formation of ectopic axon growth (Lundquist, 2003). Calcium-dependent regulation of Rho GTPases triggers turning of nerve growth cones (Jin *et al.*, 2005). The regulators of the Rho GTPases, GTPase-activating proteins and guanine exchange factors, play important roles in axon guidance. Cross GTPase-activating proteins (CrGAPs) are involved in Robo-mediated repulsive axon guidance. Too much or too little CrGAP activity leads to defects in Robo-mediated repulsion at the midline. CrGAP directly interacts with Robo both biochemically and genetically and acts as a GTPase-activating protein specifically for Rac to regulate midline crossing (Hu *et al.*, 2005).

H. Extracellular Matrix Molecules

The extracellular matrix (ECM) is an important source of extrinsic cues that influence the response of growth cones to guidance cues (Condic *et al.*, 1999; Diefenbach *et al.*, 2000; Hopker *et al.*, 1999; Nguyen-Ba-Charvet *et al.*, 2001). They can act to promote or inhibit neurite outgrowth and modulate the response of axons to particular guidance cues. Laminin, tenascin, collagen, fibronectin, and a number of proteoglycans have been implicated in modulating axonal outgrowth. For example, laminin can promote, while tenascin can inhibit, neurite extension. Receptors for ECM molecules include integrins as well as Ig family members. For example, Ig CAM F3 can function as a receptor for a type of tenascin. The laminin family and its receptors are one of the best-studied examples of ECM molecules with regard to neuronal development. The laminins are heterotrimers, in which different subunits combine to form at least 10 different isoforms with growth-promoting or -inhibiting effects depending on the cell type. The axonal receptors for the laminins are the integrins. Integrins are heterodimers whose subunit composition determines their laminin binding specificity. The integrins link the ECM signals to the cytoskeleton and various

signal transduction pathways. The exact role of laminins and other ECM molecules in neuronal development is not clear, although most evidence suggests a role in axonal guidance. Integrin signaling regulates cytoskeletal dynamics, adhesion, and migration events, through associated proteins such as talin, vinculin, integrin-linked kinase (ILK), focal adhesion kinase (FAK), paxillin, p130Cas, Abl kinase, and many other signaling or cytoskeletal proteins (Hynes, 2002).

In the developing nervous system, proteoglycans predominantly carry either chondroitin sulfate or heparan sulfate glycosaminoglycans (GAGs) (Bovolenta and Fernaud-Espinosa, 2000). Heparan sulfate proteoglycans (HSPGs) are a group of extracellular and cell surface proteins essential for proper axonal pathfinding during nervous system development (Bulow and Hobert, 2004; Walz *et al.*, 1997; Wang and Denburg, 1992), and it is increasingly evident that the major mechanism by which HSPGs influence axon pathfinding is by regulating the function of axon guidance cues. HSPGs affect several axon guidance cues, including fibroblast growth factor (FGF), heparin-binding growth associated molecule (HB-GAM), Slits, and Anosmin/Kallman syndrome gene (KAL-1) (Bulow and Hobert, 2004; Hu, 2001; Inatani *et al.*, 2003; Irie *et al.*, 2002; Johnson *et al.*, 2004; Kinnunen *et al.*, 1998; Steigemann *et al.*, 2004; Walz *et al.*, 1997). Chondroitin sulfate proteoglycans (CSPGs) are a heterogeneous set of proteins bearing GAGs of the chondroitin sulfate class (Lander, 1998). The CSPGs are also ECM molecules involved in the regulation of axon growth as demonstrated by *in vitro* studies on CSPGs such as NG2 (Dou and Levine, 1994), neurocan, and phosphacan (Margolis *et al.*, 1996). They influence the behavior of neuronal growth cones during development and, importantly, following CNS injury (Bovolenta and Fernaud-Espinosa, 2000; Morgenstern *et al.*, 2002). CSPGs are known to modulate the response of growth cones to other matrix components such as laminin (Condic *et al.*, 1999). This raises the possibility that CSPGs are components of the developmental environment capable of regulating how growth cones respond to surrounding guidance cues. The biological activity of CSPGs may also be determined by distinct proteins that bind to glycosaminoglycans and interact with receptors on the surface of neuronal growth cones (Anderson *et al.*, 1998; Brittis and Silver, 1994; Emerling and Lander, 1996; Golding *et al.*, 1999). Although CSPGs are known to interact with growth factors, adhesion molecules, and other matrix components, the specific binding proteins capable of mediating the effects of CSPGs on neuronal growth cones remain to be identified (Bovolenta and Fernaud-Espinosa, 2000; Morgenstern *et al.*, 2002).

A recent study showed that the thrombospondin repeats of Sema5A physically interact with the glycosaminoglycan portion of both CSPGs and HSPGs. CSPGs function as bound localized extrinsic cues that convert Sema5A from an attractive to an inhibitory guidance cue. Therefore,

glycosaminoglycans provide a molecular mechanism for CSPG-mediated inhibition of axonal extension. Further, axonal HSPGs are required for Sema5A-mediated attraction, suggesting that HSPGs are components of functional Sema5A receptors. Therefore, the nature of a growth cone's response to Sema5A depends on the types of sulfated proteoglycans present in the developmental environment (Kantor *et al.*, 2004).

I. Adhesion Molecules

Several cell adhesion molecules (CAMs) of the Ig superfamily have also been implicated in regulating axon guidance at the midline, including mammalian L1CAM, NrCAM, and TAG-1 (mammalian ortholog of chick Axonin-1). The superfamily includes several subfamilies that are found in a number of tissues during development and in the adult (reviewed in Edelman and Crossin, 1991). Homologues of most of these have also been found in invertebrate animals. Each CAM has an extracellular region containing six Ig domains, as well as two (NrCAM), four (L1-CAM), or five (TAG-1) fibronectin type III extracellular domains (Walsh and Doherty, 1997) (Fig. 9). The cytoplasmic domain is highly conserved among individual members of the L1 subfamily and between invertebrate and vertebrate species (Hortsch, 1996). L1 is linked to the cytoskeleton through two regions in the cytoplasmic domain. An ankyrin binding site is located in the C terminus of the cytoplasmic domain (Davis and Bennett, 1994). CAMs,

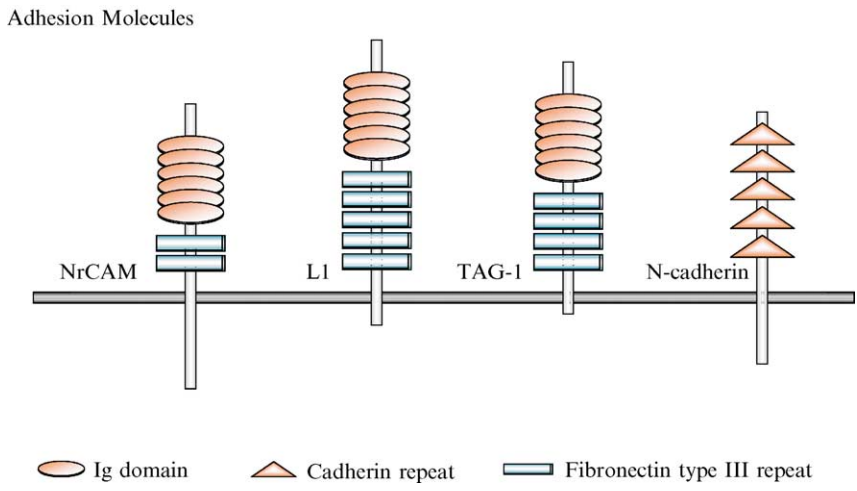


Figure 9 Adhesion molecules.

including N-CAM, L1, and N-cadherin, promote neurite outgrowth (Doherty *et al.*, 1989; Doherty *et al.*, 1990).

L1 is one of the most intensely studied adhesion molecules expressed in the developing central and peripheral nervous system (Kamiguchi *et al.*, 1998). L1 is important in neuronal migration, axon growth, guidance, fasciculation, and synaptic plasticity. L1 is also expressed in non-neuronal cells such as the immune system, kidney, pigment cells, and a variety of cancers. Commissural axons extending toward the ventral midline of the rodent spinal cord express TAG-1, but not L1, while axonal segments on the contralateral side of the floor plate express L1, but not TAG-1 (Dodd *et al.*, 1988; Imondi *et al.*, 2000; Tran and Phelps, 2000). Consistent with an altered-responsiveness mechanism, it has been postulated that the switch in expression from TAG-1 to L1, presumably triggered by contact with the floor plate, delays the rostral turn exhibited by commissural axons until after they cross the floor plate (Dodd *et al.*, 1988). NrCAM, another commissural axon-associated IgCAM, is expressed at low levels on commissural axons both as they extend toward the floor plate on the ipsilateral side of the midline and as they project in the longitudinal direction on the contralateral side of the floor plate in the chick and mouse spinal cord (Matise *et al.*, 1999; Stoeckli and Landmesser, 1995).

Knockouts of L1CAM (Cohen *et al.*, 1998; Dahme *et al.*, 1997; Fransen *et al.*, 1998) display a reduced corticospinal tract, an abnormal pyramidal decussation, a decreased axonal association with non-myelinating Schwann cells, ventricular dilatation, and hypoplasia of the cerebellar vermis. Demyanenko and colleagues (1999) reported abnormal morphogenesis of cortical dendrites, showing that pyramidal neurons in layer V exhibited undulating apical dendrites that did not reach layer I. They also found that L1 mutants had a smaller hippocampus with fewer pyramidal and granule cells (Demyanenko *et al.*, 1999) and an altered distribution of dopaminergic neurons in the brain of L1 null mice (Demyanenko *et al.*, 2001). There is also a reduced size of the corpus callosum because of the failure of many callosal axons to cross the midline, as well as the formation of other commissural tracts in the brain. L1 has been shown to interact with neuropilin via the first Ig domain (Castellani *et al.*, 2002) and to participate in Sema3a signaling (Castellani *et al.*, 2000) to mediate axonal repulsion.

V. Axon Guidance Mechanisms at the Midline of the Nervous System

A. Commissural Axons in Spinal Cord

The spinal cord has proven to be a useful system for identifying molecules that guide axons to their appropriate targets. During spinal cord development, commissural neurons, which differentiate in the dorsal neural tube,

send axons that project toward and subsequently across the floor plate, forming axon commissures (Colamarino and Tessier-Lavigne, 1995). These commissural axons project toward the midline in part because they are attracted by Netrin-1 (Kennedy *et al.*, 1994; Placzek *et al.*, 1990; Serafini *et al.*, 1996, 1994; Tessier-Lavigne *et al.*, 1988b). Once on the contralateral side, axons are no longer attracted but are repelled by Slit expressed by ventral midline cells (Brose and Tessier-Lavigne, 2000). This change in the response of axons is due to the silencing of Netrin-1 attraction by signaling through DCC interacting with the Slit receptor, Robo (Stein and Tessier-Lavigne, 2001). In *Netrin-1* or *DCC* mutant mice, many commissural axon trajectories fail to invade the ventral spinal cord and are misguided (Fazeli *et al.*, 1997; Serafini *et al.*, 1996). However, some of them do reach the midline, indicating that other guidance cues cooperate with Netrin-1 to guide these axons (Serafini *et al.*, 1996). Sonic hedgehog (Shh), a morphogen secreted by the floor plate, functions as a gradient signal for the generation of distinct classes of ventral neurons along the dorsoventral axis (Ingham and McMahon, 2001; Jessell, 2000; Marti and Bovolenta, 2002). Shh is an axonal chemoattractant that provides the Netrin-1-independent chemoattractant activity of the floor plate. Shh collaborates with Netrin-1 in commissural axon attraction *in vitro* and is required for normal guidance of these axons *in vivo* (Charron *et al.*, 2003). Several studies show that other members of the morphogen family, Wnt and BMP, are also involved in the spinal cord development. A directed source of Wnt4 protein attracts postcrossing commissural axons and Frizzled3 mutant mice display anterior-posterior guidance defects after midline crossing. This indicates that Wnt-Frizzled signaling guides commissural axons along the anterior-posterior axis of the spinal cord (Lyuksyutova *et al.*, 2003). Bmp7, Bmp6, and growth differentiation factor 7 (Gdf7) are expressed by the roof plate and are potential dorsal repellent cues for commissural axons (Augsburger *et al.*, 1999).

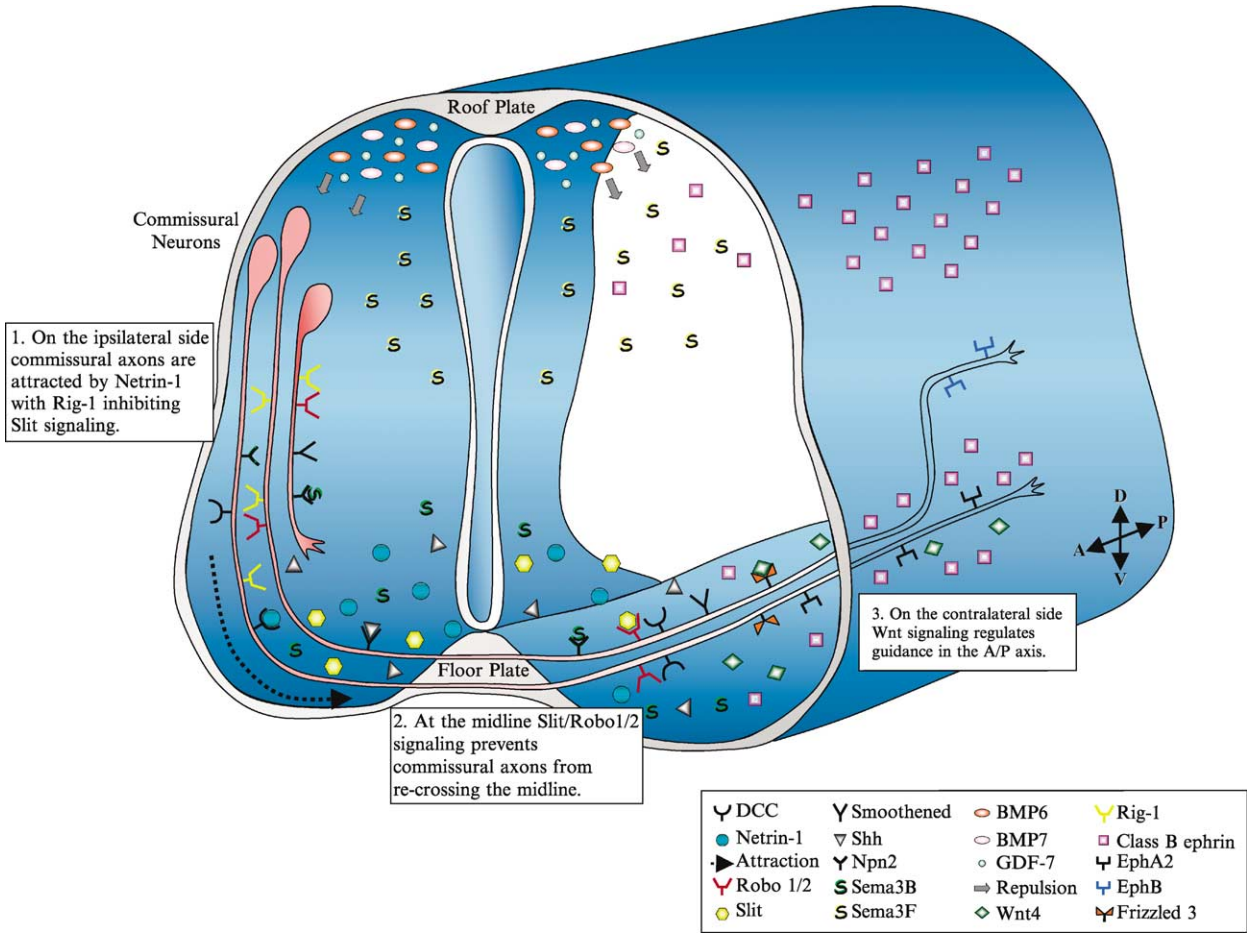
The *Slit* genes are also expressed in the floor plate at the ventral midline of the spinal cord, and *Robo1* and *2* are expressed in regions that include commissural neuron cell bodies (Brose *et al.*, 1999; Itoh *et al.*, 1998; Kidd *et al.*, 1998; Li *et al.*, 1999). *In vitro* commissural axons are repelled by Slit2 only after they have crossed the floor plate (Zou *et al.*, 2000). In *Slit1/Slit2* double mutant mice, although the formation of several major forebrain tracts (corticofugal, callosal, and the thalamocortical tracts) and the optic chiasm are defective (Bagri *et al.*, 2002; Plump *et al.*, 2002), no obvious commissural axon guidance defects were observed in the spinal cord (Plump *et al.*, 2002). Another member of the Slit family, Slit3, is also expressed by floor plate cells (Brose *et al.*, 1999), and analysis of triple Slit mutant revealed that many commissural axons stalled at the floor plate and failed to cross (Long *et al.*, 2004). This indicates that Slits contribute to the repulsion of axons away from the midline. Rig-1 is a divergent member of

the Robo family (Yuan *et al.*, 1999) that is highly expressed before midline crossing and is downregulated after crossing (Sabatier *et al.*, 2004). Rig-1 prevents commissural axons from sensing Slit in the floor plate through their cognate receptor Robo1 as they grow toward the floor plate, allowing them to enter and cross to the contralateral side. At the midline, the downregulation of Rig-1 protein expression helps the axons to sense the floor plate as a repulsive environment, thus preventing them from re-crossing the midline (Sabatier *et al.*, 2004). Commissural axons also express another receptor, Neuropilin-2, mediating the repulsive effects of Sema3B, found in the floor plate, and Sema3F, expressed widely in the spinal cord, except in the floor plate (Zou *et al.*, 2000; Fig. 10). Analysis of homozygous *neuropilin-2* mutant mice shows disorganized axons at the midline while crossing (Zou *et al.*, 2000), suggesting a role for neuropilin-2 in commissural axon pathfinding.

Once the axons have crossed the midline, they execute a rostral turn at the contralateral floor plate margin and extend for a short distance within the ventral funiculus, a longitudinal fiber tract that forms in close apposition to the floor plate (Bovolenta and Dodd, 1990). EphA2 and EphB expression is upregulated on contralateral commissural axons (Brittis *et al.*, 2002; Imondi *et al.*, 2000), and class B ephrins are expressed in the floor plate as well as in the dorsal part of the spinal cord. A subset of decussated commissural axons takes a more dorsal trajectory before turning at the border of class B ephrin expression, indicating a role in excluding these axons from the dorsal spinal cord (Imondi and Kaprielian, 2001; Fig. 10).

B. Guidance of Cortical Axons at the Midline

Contralateral cerebral cortical projections through the corpus callosum integrate sensory and motor information between the two brain hemispheres. In split-brain animals and in people whose corpus callosum has been severed, interhemispheric transfer of sensory and motor information is deficient (Gazzaniga, 1995). In these individuals, visual and tactile information presented to one hemisphere is not available for analysis by the other hemisphere. In addition, perceptual interactions between the two hemispheres are absent in these individuals. These observations, pioneered by Roger Sperry in the 1960s (Sperry, 1968, 1982), defined the critical roles of contralateral cortical projections in human consciousness and behavior. Data in humans and in mice suggest the possibility that different mechanisms may regulate the development of the corpus callosum across its rostrocaudal extent (Richards *et al.*, 2004). The complex developmental processes required for formation of the corpus callosum may provide some insight into why such a large number of human congenital syndromes are associated with agenesis of this structure (Richards *et al.*, 2004). Anatomical studies


Roof Plate
Commissural Neurons
1. On the ipsilateral side commissural axons are attracted by Netrin-1 with Rig-1 inhibiting Slit signaling.
Floor Plate
2. At the midline Slit/Robo1/2 signaling prevents commissural axons from re-crossing the midline.
3. On the contralateral side Wnt signaling regulates guidance in the A/P axis.
D
P
A
V
DCC
Netrin-1
Attraction
Robo 1/2
Slit
Smoothened
Shh
Npn2
Sema3B
Sema3F
BMP6
BMP7
GDF-7
Repulsion
Wnt4
Rig-1
Class B ephrin
EphA2
EphB
Frizzled 3

have demonstrated that the majority of contralaterally projecting (callosal) neurons are located in layers 2/3 and 5 (Innocenti, 1986; Wise and Jones, 1976). In rodents callosal axons project to corresponding, homotopic areas in the contralateral cortex. During development, callosal axons grow ventrally to the intermediate zone (the future cortical white matter) and then turn medially, cross the midline, and re-enter the appropriate contralateral cortical area to form synapses with their targets (Fig. 11). The development of the corpus callosum depends on guidance by midline glial populations, and their expression of specific molecules, and fasciculation pioneering axons derived from neurons in the cingulate cortex (as described in Section III.A.2). Four midline populations at the corticoseptal boundary have been described: the glial wedge, the indusium griseum glia (Shu and Richards, 2001; Shu *et al.*, 2003c), the midline zipper glia (Silver *et al.*, 1993), and the subcallosal (glial) sling (Silver *et al.*, 1982) (see Section III.B). The sling is a glial fibrillary acidic protein (GFAP)-negative population of cells (in rodents) with neuronal properties (Shu *et al.*, 2003b) that migrates from the lateral sub-ventricular zone to underlie the developing corpus callosum (Silver *et al.*, 1982). Both ablation and rescue experiments (Silver and Ogawa, 1983; Silver *et al.*, 1982) have shown that the glial sling is required for the development of the corpus callosum. The indusium griseum glia and the midline zipper glia have many phenotypic and molecular characteristics in common, indicating that they may represent a common population of glia that becomes spatially distinct by the formation of the corpus callosum (Shu *et al.*, 2003c). The glial wedge is part of the radial glial scaffold (Shu *et al.*, 2003c) and, together with the indusium griseum glia, expresses Slit2. In the brain, unlike the spinal cord, Slit2 mediates both precrossing and postcrossing axonal guidance (Shu *et al.*, 2003d). Robo1 and Robo2 mRNAs are expressed in the neocortex during callosal axon targeting (Shu and Richards, 2001), and Robo proteins are expressed on callosal axons (Sundaresan *et al.*, 2004). In the Slit2 mutant, the corpus callosum fails to form. Instead, axons grow into large ectopic bundles of fibers on either side of the midline that resemble Probst bundles (Bagri *et al.*, 2002). Taken together, these data

Figure 10 Molecules involved in the guidance of commissural axons in the spinal cord. Commissural neurons send their axons toward and across the midline. Bmp6 and 7 and GDF-7 expressed by the roof plate act as dorsal repellents for commissural axons. Axons express DCC, Robo1/2, Smoothened, and Npn2. The axons are attracted by Netrin-1 and Shh but initially are not responsive to Slit because Rig-1 inhibits Slit/Robo1/2 signaling. Sema/Npn2 signaling is required to avoid inappropriate targeting. Once the axons cross the midline, they are then repelled by Slit, expressed by the floor plate, and lose their attraction to Netrin-1, allowing them to leave the floor plate and preventing them from re-crossing the midline. Wtn4/Frizzled3 signaling regulates guidance in the anterior-posterior axis. A subset of commissural axons expressing EphB takes a more dorsal trajectory but grows between regions of Class 3 ephrin-B expression both dorsally and ventrally.

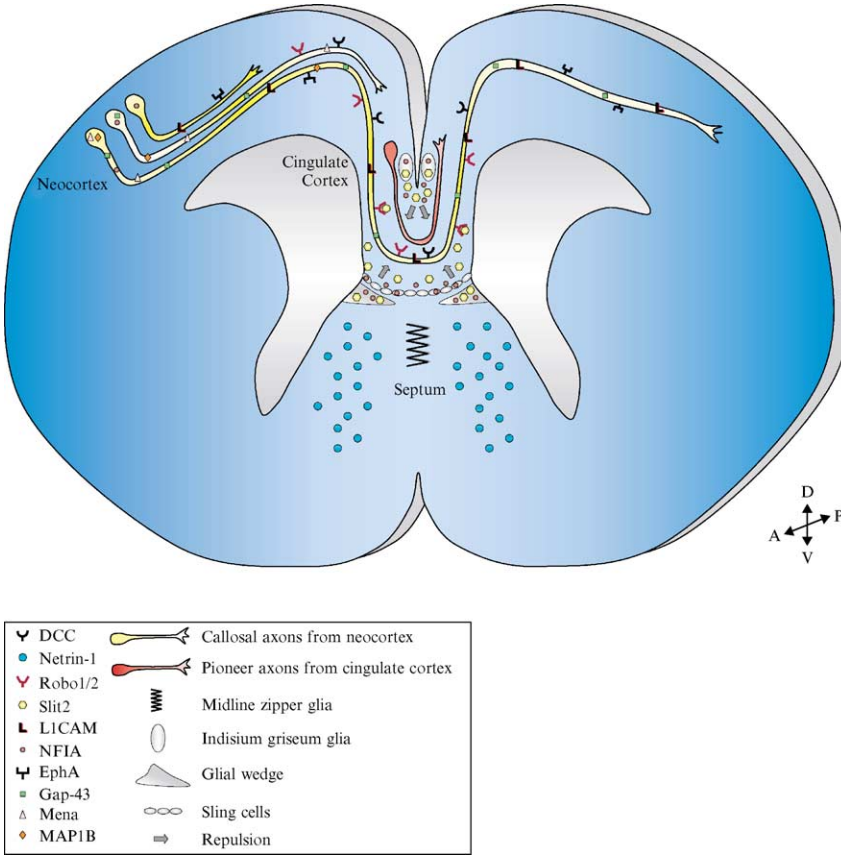


Figure 11 Molecules involved in the formation of the cortical commissural axons. Axons from the cingulate cortex pioneer a path across the midline. They are followed by neocortical callosal axons that probably fasciculate with them to cross the midline. Slit2, expressed by the glial wedge and the indusium griseum glia, provides a surround repulsion mechanism that keeps callosal axons within the tract, causing them to turn and cross the midline and preventing them from entering the septum. After axons have crossed the midline, Slit2 then repels the postcrossing axons away from the midline area. Callosal axons express several different molecules, including DCC, Robo1/2, EphA, NFIA, Gap-43, L1, Mena, and MAP1B. Netrin-1 is expressed within the septum under the corpus callosum; thus, unlike commissural axons in the spinal cord, callosal axons do not grow through the region of Slit and Netrin expression. NFIA is present in the sling cells, glial wedge, and the indusium griseum. Mouse mutants for DCC, Netrin-1, EphA, NFIA, Gap-43, L1, Mena, and MAP1B have defects in callosal formation (see Section V.B for more details) but how these genes regulate callosal axon pathfinding has not yet been elucidated.

suggest an important role for Slit/Robo signaling in corpus callosum formation.

In the mammalian brain, Netrin-1 mutants and DCC knockout mice do not develop a corpus callosum or a hippocampal commissure and have a greatly reduced or absent anterior commissure (Fazeli *et al.*, 1997; Serafini *et al.*, 1996). In the developing forebrain, Netrin-1 has been shown to attract laterally directed cortical axons *in vitro* (Metin *et al.*, 1997; Richards *et al.*, 1997), indicating that these molecules are important for additional axonal guidance systems in the forebrain other than commissural axon guidance. Netrin-1 acts *in vitro* as an attractant and growth promoter for dorsal thalamic axons and is required for the proper development of the thalamocortical axon (TCA) projection *in vivo* (Braisted *et al.*, 2000). DCC protein is expressed predominantly in large fiber tracts such as the lateral olfactory tract, the internal capsule, the corpus callosum, the anterior commissure, the fimbria/fornix, the fasciculus retroflexus, and the stria medularis (Shu *et al.*, 2000), as well as in dorsal thalamus (Braisted *et al.*, 2000). DCC knockout mice have defects in multiple commissures, including the corpus callosum, the hippocampal commissure, and the anterior commissure (Fazeli *et al.* 1997). The basilar pons is absent in both Netrin-1 and DCC knockout mice (Fazeli *et al.*, 1997; Yee *et al.*, 1999).

The nuclear factor I (Nfi) family of transcription factors regulates both adenoviral DNA replication and viral and cellular gene expression, including the control of olfactory-specific genes (Baumeister *et al.*, 1999; Behrens *et al.*, 2000; Gronostajski *et al.*, 1985; Hennighausen *et al.*, 1985; Leegwater *et al.*, 1985; Nagata *et al.*, 1982, 1983; Nowock *et al.*, 1985). The Nfi family is made up of four members, Nfia, Nfib, Nfic, and Nfix. The Nfia mutant exhibits both agenesis of the corpus callosum and a reduction in GFAP expression (das Neves *et al.*, 1999). Nfia is expressed in midline glial structures, and the development of these structures is severely impaired in Nfia mutant mice. These data indicate that Nfia regulates both commissural development and the development of midline glia (Shu *et al.*, 2003a). Nfib mutant mice also display agenesis of the corpus callosum and abnormalities in midline glial development, as well as enlargement of the lateral ventricles (Steele-Perkins *et al.*, 2005).

In knockouts of the L1 gene (Cohen *et al.*, 1998; Dahme *et al.*, 1997; Fransen *et al.*, 1998) the corpus callosum failed to form properly due to the failure of many callosal axons to cross the midline (Demyanenko *et al.*, 1999, 2001). These findings suggest a variety of biological roles for L1 that are critical in brain development in different brain regions. Nr-CAM, a member of the L1 subfamily of cell adhesion molecules, is not expressed on callosal axons until postnatal day zero (P0), suggesting that Nr-CAM may be involved in the later stages of axonal growth or tract maintenance (Lustig *et al.*, 2001).

Mice lacking p35, an activator of cdk5 in the CNS, exhibit defects in a variety of CNS structures, most prominently characterized by a disruption in the laminar structure of the neocortex (Chae *et al.*, 1997). In these mutant mice, the corpus callosum appears bundled at the midline, but dispersed lateral to the midline. After crossing the midline, cortical axons defasciculate prematurely from the corpus callosum and take similarly oblique paths through the cortex. These results suggest that defective axonal fasciculation and guidance may be primary responses to the loss of p35 in the cortex (Kwon *et al.*, 1999).

In EphA5 mutant mice, callosal axons failed to grow into the corpus callosum, indicating that the EphA receptors and their ligands, the A-ephrins, play critical roles in the development of callosal axon projection to their contralateral targets (Hu *et al.*, 2003). EphA4 is expressed in the developing corpus callosum, with an interesting differential expression of EphA4 within different parts of the corpus callosum. In rostral regions, the entire corpus callosum was EphA4 positive whereas more caudally (around the hippocampal commissure) EphA4 was restricted to the most dorsal part of the corpus callosum (Greferath *et al.*, 2002). EphB2 (Nuk) and EphB3 (Sek4) mutant mice have been described (Henkemeyer *et al.*, 1996; Orioli *et al.*, 1996). EphB2 and EphB3 are members of the Eph-related family of receptor protein-tyrosine kinases. These receptors interact with a set of cell surface ligands that have recently been implicated in axon guidance and fasciculation. Whereas mice deficient in EphB2 exhibit defects in pathfinding of anterior commissure axons, EphB3 mutants have defects in corpus callosum formation. The phenotype in both axon tracts is markedly more severe in EphB2/EphB3 double mutants, indicating that the two receptors act in a partially redundant fashion (Orioli *et al.*, 1996).

MAP1B, a microtubule-associated protein, is expressed in axons, dendrites, and growth cones throughout the CNS during development. MAP1B is implicated in the crosstalk between microtubules and actin filaments. Homozygous MAP1B mutant mice display agenesis of the corpus callosum (Meixner *et al.*, 2000). A recent study showed that MAP1B phosphorylation is controlled by Netrin-1 (Del Rio *et al.*, 2004). Map1B mutant mice have severe abnormalities, similar to those described in netrin-1-deficient mice, in axonal tracts and in the pontine nuclei. These data indicate MAP1B may be a downstream effector in the Netrin-1-signaling pathway (Del Rio *et al.*, 2004).

A number of other genes are associated with agenesis of the corpus callosum in mice (reviewed in Richards *et al.*, 2004). The most common phenotype observed when cortical axons fail to reach the midline was that the axons do not stop growing but instead form swirled ipsilateral bundles of axons, called Probst bundles. Probst bundles form in mutants such as *Vax-1* (Bertuzzi *et al.*, 1999), *Gap-43* (Shen *et al.*, 2002), or heparan sulfate (Inatani

et al., 2003), indicating that these genes may regulate callosal axon guidance at the midline. However, in *GAP-43* mutant mice, callosal axons respond normally to Slit-2, although glial abnormalities may contribute to the phenotype (Shen *et al.*, 2002).

C. Guidance of Retinal Ganglion Cell Axons at the Optic Chiasm

In animals with binocular vision (such as mammals), retinal ganglion cell (RGC) axons originating from the nasal retina cross the midline to project into the contralateral optic tract, while a population of RGC axons from the temporal retina do not cross, but project away from the midline into the ipsilateral optic tract (Fig. 12). In mouse, ipsilaterally projecting RGCs are found in the ventro-temporal crescent of the retina, whereas contralaterally projecting RGCs are found throughout the retina (Guillery *et al.*, 1995; Mason and Sretavan, 1997; Sretavan, 1993).

After retinal ganglion cell axons exit each eye at the optic nerve head, forming the optic nerve, they traverse the ventral diencephalon toward the midline. Axons from the two eyes cross over each other to form the chiasm (X shape). The proportion of uncrossed to crossed retinal fibers varies across species. The ipsilateral projection represents about 40% of all RGCs in humans (Kandel *et al.*, 2000), less than 15% in ferrets (Cucchiaro, 1991; Thompson and Morgan, 1993), and about 3–5% in mice (Rice *et al.*, 1995). Adult birds and fish do not have an ipsilateral projection (O'Leary *et al.*, 1983) and thus lack binocular vision.

Neuronal cells within the chiasm are postulated to provide guidance cues for the earliest axons (Marcus and Mason, 1995; Sretavan *et al.*, 1994). When these neurons are destroyed by complement-mediated cytolysis, the growth of all axons entering the chiasm is halted (Sretavan *et al.*, 1995). Later axons, however, derive guidance cues from a midline palisade of radial glia. The cells in this region provide a generalized negative signal, as the growth cones of both crossed and uncrossed axons pause when entering this region *in vivo* (Godement, 1994) and *in vitro* (Mason and Wang, 1997; Wang *et al.*, 1996). After the optic chiasm, axons then continue through the optic tract to their targets: the superior colliculus in mammals or the optic tectum in fish, frogs, or birds. The main visual nuclei that receive retinal input are the lateral geniculate nucleus (LGN), the superior colliculus (SC), and the pretectal nuclei (Wassle, 1982). They also project to the superchiasmatic nucleus and the accessory optic system (Zhang and Hoffmann, 1993). Retinal axons express DCC (as well as the repulsive receptor UNC-5), but Netrin is absent from the chiasm region (Anderson and Holt, 2002; Deiner and Sretavan, 1999; Shewan *et al.*, 2002). Instead, Netrin is expressed at the optic nerve head, and in Netrin or DCC mutants RGC axons fail to exit this

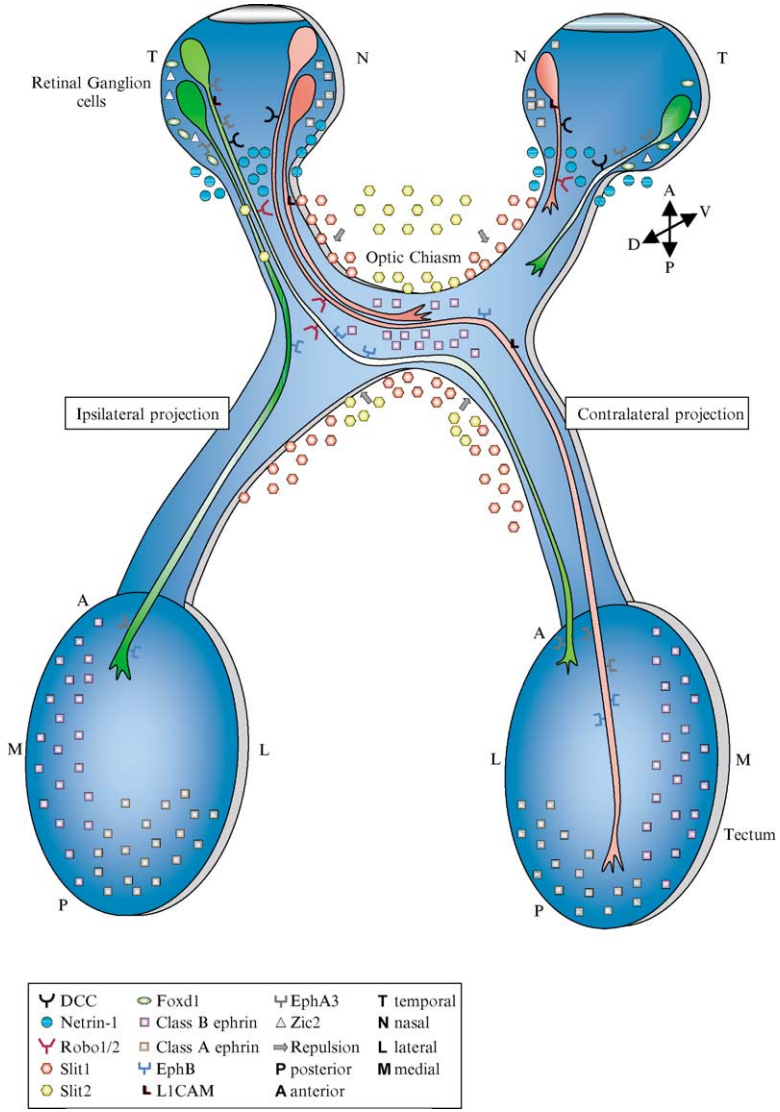


Figure 12 Molecules involved in the guidance of RGC axons to the tectum. The majority of RGC axons projects into the contralateral optic tract, while a small population of temporal RGC axons does not cross and projects ipsilaterally in mice. A diverse group of molecules acts to guide the RGC axons toward their final target in the tectum. Netrin-1 is expressed in the optic nerve head and Slits are expressed in the optic chiasm region, channeling the axons through the tectum and determining the position of the chiasm. RGC axons express DCC, Robo1/2, L1, and EphB and, as they enter the tectum, they express EphA3. Class B ephrins are expressed in the medial part of the tectum and mediate dorsoventral targeting, whereas Class A ephrins are expressed in the posterior tectum and mediate anterior-posterior topographic targeting.

region (Deiner *et al.*, 1997). In the retina, *robo2* is expressed by RGCs before any axons have reached the ventral midline of the diencephalon and continues to be strongly expressed during later stages of development. In contrast, *robo1* is not detected until after a number of axons have started to cross the midline and then only in a subset of cells. This suggests that Robo2 is likely to be the principle receptor in RGC axon guidance (Erskine *et al.*, 2000). Two slit genes are expressed around the chiasm: Slit1 and Slit2 in mouse, Slit2 and Slit3 in zebrafish. Furthermore, Slit1 and Slit2 both repel retinal axons in culture (Erskine *et al.*, 2000; Niclou *et al.*, 2000; Ringstedt *et al.*, 2000). As shown by experiments in zebrafish and mouse, Robo/Slit signaling controls the formation of the optic chiasm (Fricke *et al.*, 2001; Hutson and Chien, 2002; Plump *et al.*, 2002). Zebrafish mutant for the astray/robo2 receptor show multiple guidance errors near the chiasm, including ipsilateral, anterior, and retinoretinal projections. Slit1 or Slit2 single knockout mice have little or no retinal projection phenotype, presumably because of the partial overlap in the expression of the two genes. However, Slit1 and Slit2 double knockouts show a strong phenotype, most notably the expansion of the chiasm more anteriorly. Developmental and time-lapse analyses in zebrafish embryos show that, even in wild-type animals, RGC growth cones occasionally leave their pathway and misproject, but these errors are quickly corrected (Hutson and Chien, 2002). In astray mutants, many more errors occur and persist. Thus, Slit/Robo signaling has two functions: preventing errors in the first place and correcting them if they do occur (Rasband *et al.*, 2003).

Optic nerve fibers grow out from the eye in fasciculated bundles, with contact between them mediated in part by membrane glycoproteins. The first of these glycoproteins to be identified was neural cell adhesion molecule (NCAM) (Jessell, 1988). NCAM plays a crucial role in axonal fasciculation and substrate adhesion. The localization of the cell adhesion molecules L1, NCAM, and myelin-associated glycoprotein (MAG) was studied in the developing and adult mouse optic nerve and retina. At all stages of development, NCAM is expressed by fasciculating axons, growth cones, and their contact sites with glial cells, and contacts between glial cells. MAG is first associated with the endoplasmic reticulum and Golgi apparatus in oligodendrocytes and then moves at cell surface. L1 is expressed by optic axons that are grouped into fascicular bundles and by their growth cones, but not by the growth cones that contact glial cells (Bartsch *et al.*, 1989). L1 was differentially expressed on unmyelinated axons, but absent on myelinated axons. This finding supports the notion that L1 may be involved in the stabilization of axonal fascicles but not of axon-myelin contacts (Bartsch *et al.*, 1989). Recent reports have shown that the absence of L1 in the optic pathway in L1 knockout mice has no obvious effect on the development pattern of axon divergence in the chiasm (Cohen *et al.*, 1998; Demyanenko and Maness, 2003). L1 and the polysialic acid-associated form of NCAM

(PSA-NCAM) are dynamically expressed in a regionally specific pattern in the retinotectal pathway (Chung *et al.*, 2004). At the chiasm, the level of L1 expression is high, whereas that of PSA-NCAM is low. However, within the tract, intense expression of both molecules is found predominantly on axons from the dorsal but not ventral retina. These changes are observed when axons arrive at the junction of the chiasm and the optic tract, indicating a site-specific switch in expression of cell adhesion molecules on the optic axons. Moreover a population of PSA-NCAM-rich cells also projects axons to the TPOC. These results suggest that CAM expression on the optic axons may control formation of the partial retinotopic axon order within the optic tract (Chung *et al.*, 2004).

CSPGs provide an unfavorable environment for axon growth and have been implicated in the changing patterns of fiber order in the developing retinotectal pathway (Brittis and Silver, 1995). In mouse, CSPGs are expressed by an early population of neurons in the ventral diencephalon, and enzymatic removal of the chondroitin moieties affects retinal ganglion cell guidance (Chung *et al.*, 2000a,b). CSPGs are expressed in the retina as well (Chung *et al.*, 2000a) and are also important for maintaining fiber order as axons approach the chiasm (Leung *et al.*, 2003).

Heparan sulfates are also involved in the formation of the optic chiasm (Inatani *et al.*, 2003). Analysis of mutant mice for EXT1, an enzyme indispensable for HS synthesis, revealed that retinal axons projected ectopically into the contralateral optic nerve, similar to Slit1/Slit2 double-knockout mice (Plump *et al.*, 2002).

B-class ephrins are required for the sorting of axons at the optic chiasm. In *Xenopus laevis*, ephrin-B2 is present at the chiasm coincident with the formation of the uncrossed component at metamorphosis, and premature misexpression of ephrin-B2 in the ventral diencephalon induced an ectopic ipsilateral projection (Nakamura *et al.*, 2000). EphB receptors are expressed in the retina (Birgbauer *et al.*, 2000; Braisted *et al.*, 1997; Hindges *et al.*, 2002; Mann *et al.*, 2002b; Williams *et al.*, 2003). A receptor for ephrin-B2, EphB1, is found exclusively in regions of retina that give rise to the ipsilateral projection. EphB1 null mice exhibit a dramatic reduction in the ipsilateral projection, suggesting that this receptor contributes to the formation of the ipsilateral retinal projection, most likely through its repulsive interaction with ephrin-B2 (Williams *et al.*, 2003).

Several regulatory genes expressed in the developing retina have been reported to play a role in retinal axon guidance at the optic chiasm. Mice lacking Vax1, Vax2, Pax2, or Brn3b exhibit different defects in retinal axon pathfinding at the chiasm (Barbieri *et al.*, 2002; Bertuzzi *et al.*, 1999; Mui *et al.*, 2002; Torres *et al.*, 1996; Wang *et al.*, 2002b). It has also been shown that mutual regulation of Pax-2 and Shh are important for the formation of the chiasm region (Alvarez-Bolado *et al.*, 1997). Overexpression of Pax-2 affects

axon navigation through the chiasm and in the ascending postchiasmatic pathway, including the optic tract up to the tectum (Thanos *et al.*, 2004).

The zinc finger transcription factor Zic2 is expressed in retinal ganglion cells (Fig. 12). Loss- and gain-of-function analyses indicate that Zic2 is necessary and sufficient to regulate RGC axon repulsion by cues at the optic chiasm and to determine the ipsilateral projection (Herrera *et al.*, 2003).

The winged helix transcription factor Foxd1 (previously known as BF-2, brain factor 2) is expressed in the ventrotemporal retina, as well as in the ventral diencephalon during the formation of the optic chiasm. Both retinal development and chiasm morphogenesis are disrupted in embryos lacking Foxd1. In the Foxd1-deficient retina, proteins designating the ipsilateral projection, such as Zic2 and EphB1, are missing. In addition, in the Foxd1-deficient ventral diencephalon, Foxg1 expression invades the Foxd1 domain, Zic2 and Islet1 expression are minimized, and Slit2 expression prematurely expands, changes that could contribute to axon projection errors. Foxd1 plays a dual role in the establishment of the binocular visual pathways: first, in specification of the ventrotemporal retina, acting upstream of proteins directing the ipsilateral pathway, and second, in the patterning of the developing ventral diencephalon where the optic chiasm forms (Herrera *et al.*, 2004).

After forming the optic chiasm, RGC axons extend laterally and dorsally to establish the optic tract along the lateral wall of the diencephalon to reach visual targets in the thalamus and midbrain.

VI. Axon Guidance in the Retinotectal System

The retinotectal projection is one of the best-studied model systems for the examining of the mechanisms regulating precise topographic connectivity in the embryonic CNS. In 1963, Roger Sperry showed that after optic nerve section, retinal ganglion cell axons regenerate to their normal topographic positions in the tectum regardless of whether the entire retina is present (Attardi and Sperry, 1963). He proposed the chemoaffinity hypothesis: that each point in the target area has a unique molecular address determined by a specific distribution of cell surface molecules (Sperry, 1963). The retinotectal projection from the temporal retina is connected to the anterior tectum and the retinotectal projection from the nasal retina is connected to the posterior tectum (Fig. 12). Dorsal retina is connected to the dorsal tectum and ventral retina is connected to the ventral tectum (Holt and Harris, 1993; Mey and Thanos, 1992; Thanos and Mey, 2001; van Horck *et al.*, 2004). Time-lapse analyses of the retinotectal projection have demonstrated that retinal ganglion cell axons grow rapidly within the optic tract but move slowly after reaching the tectum, suggesting the presence of target-derived cues that suppress axonal growth (Harris *et al.*, 1987).

A combination of *in vitro* and genetic studies has revealed that the mapping of RGC axons along the anterio-posterior axis of the optic tectum is controlled in large part by the matched gradients of EphA receptor tyrosine kinases in the retina and the GPI-linked ephrin ligands in the tectum (McLaughlin *et al.*, 2003a). Activation of EphA by ligand binding leads to axon repulsion (Drescher *et al.*, 1997; Flanagan and Vanderhaeghen, 1998) and inhibition of axon branching (Yates *et al.*, 2001). Ephrin-A2 and ephrin-A5 are expressed in overlapping gradients in the tectum, while the corresponding receptors are expressed in complementary gradient across the retina (Cheng *et al.*, 1995). Axons from temporal retinal ganglion cells, where EphA expression is high, are inhibited from innervating the caudal tectum, where ephrin-A levels peak (Ruthazer and Cline, 2004). Ephrin-As control the temporal-nasal mapping of the retina in the optic tectum/superior colliculus by regulating the topographically specific interstitial branching of retinal axons along the anterior-posterior tectal axis. This branching is mediated by relative levels of EphA receptor repellent signaling (McLaughlin *et al.*, 2003a). In ephrin-A2/A5 double knockout mice the retinotectal map is severely disrupted (Feldheim *et al.*, 2000), indicating complementary functions of these two molecules in retinotectal mapping.

Members of the EphB family of receptor tyrosine kinases and their transmembrane ligands ephrin-Bs have been implicated in dorsoventral patterning of the vertebrate retinotectal projection (Flanagan and Vanderhaeghen, 1998). EphB/ephrin-B interactions mediate axon attraction (Hindges *et al.*, 2002; Mann *et al.*, 2002a). It has been shown in mice that EphB in ventral retina contributes to axon targeting to the medial part of the superior colliculus, where ephrin-B levels are high (Hindges *et al.*, 2002). In *Xenopus*, high levels of ephrin-B in dorsal RGCs guide axons to the EphB-rich ventral tectum (Mann *et al.*, 2002a). EphB/ephrin-B interactions are known to result in bidirectional signaling, characterized by signaling into cells expressing EphB receptors (i.e., forward signaling) and into cells expressing ephrin-B (i.e., reverse signaling) (Holland *et al.*, 1996). Both reverse and forward ephrin-B/EphB signaling are involved in regulating dorso-ventral topography (Hindges *et al.*, 2002; Mann *et al.*, 2002a; Pittman and Chien, 2002). EphB receptor forward signaling and ephrin-B reverse signaling mediate axon attraction to control dorsal-ventral retinal mapping along the lateral-medial tectal axis (McLaughlin *et al.*, 2003b).

VII. Axon Guidance in the Olfactory System

The sense of smell is a primal sense for humans as well as animals. For both humans and animals, it is an important means with which we sense our environment. Compared to other sensory systems, the olfactory system is

unique in that new olfactory receptor neurons are continuously produced throughout life (Graziadei and Monti Graziadei, 1978). This process is essential for the replacement of mature olfactory receptor neurons that have a limited life span. Axons derived from newly differentiating olfactory receptor neurons in the olfactory epithelium project to glomeruli in the main olfactory bulb, where they synapse on the dendrites of mitral and tufted cells, whose neurons then send axons to the primary olfactory cortex (Shipley and Ennis, 1996). Therefore, all new olfactory axons exiting the olfactory epithelium, in embryos and adults, must be guided to their specific glomerular targets in the olfactory bulb. The precise olfactory axon targeting depends on the combination of guidance cues they encounter along their pathway.

A. Guidance Cues in the Main Olfactory System

The main olfactory system is formed by the olfactory epithelium, the olfactory nerve, and the olfactory bulbs. Olfactory sensory neurons located in the epithelium of the principal nasal cavity project their axons to the main olfactory bulb (MOB), where they synapse with second-order neurons (mitral and tufted cells) within specialized compartments of neuropil called glomeruli (reviewed in Dulac, 2000; Halpern, 1987). In rodents, axons extending from the olfactory epithelium grow initially through mesenchyme rich in NCAM (Croucher and Tickle, 1989; Key and Akeson, 1990; Miragall *et al.*, 1989), retinoic acid, FGF-8 (LaMantia *et al.*, 2000, 1993), L1, laminin, and HSPG and CSPG (Gong and Shipley, 1996; Raabe *et al.*, 1997; Treloar *et al.*, 1996). Several additional candidate guidance factors have been identified in the developing and mature olfactory system, but functional evidence for their involvement in the precise connectivity of olfactory sensory neurons is limited. Directional outgrowth toward distinct regions in the developing bulb involves a variety of signaling molecules, including semaphorins and neuropilins (Kobayashi *et al.*, 1997; Pasterkamp *et al.*, 1998, 1999; Renzi *et al.*, 2000; Schwarting *et al.*, 2000; Walz *et al.*, 2002; Williams-Hogarth *et al.*, 2000), adhesion molecules (Kafitz and Greer, 1998; Puche and Key, 1996; Yoshihara *et al.*, 1997), and Eph/ephrins (Maisonpierre *et al.*, 1993; St John and Key, 2001; St John *et al.*, 2000, 2002; Zhang *et al.*, 1996). Olfactory sensory neurons expressing a given odorant receptor project with precision to specific glomeruli in the olfactory bulb, generating a topographic map. This implies a tight linkage between the choice of a specific odorant receptor and the choice of a glomerular target. Mombaerts *et al.* (1996) developed a genetic approach to visualize these projections and examine the relationship between receptor expression and axon targeting. They observed that neurons expressing P2, an olfactory receptor gene, project with precision to two of

the 1800 glomeruli within the olfactory bulb. These data provide direct support for a model in which a topographic map of the receptor topographically fixed glomeruli in the mouse olfactory bulb. Substitution of the olfactory receptor gene leads to the conclusion that the olfactory receptor may be one determinant in the guidance process (Mombaerts *et al.*, 1996; reviewed in Mombaerts, 1996). Neurons expressing different odorant receptors express different levels of ephrin-A protein on their axons. Moreover, alterations in the level of ephrin-A alter the glomerular map. Deletion of the ephrin-A5 and ephrin-A3 genes posteriorizes the glomerular locations for neurons expressing either the P2 or SR1 receptor, whereas overexpression of ephrin-A5 in P2 neurons results in an anterior shift in their glomeruli. Thus the ephrin-As are differentially expressed in distinct subpopulations of neurons and are likely to participate, along with the odorant receptors, in governing the targeting of like axons to precise locations in the olfactory bulb (Cutforth *et al.*, 2003).

Expression of dominant-negative neuropilin-1 protein causes chick olfactory neurons to enter the telencephalon prematurely (Renzi *et al.*, 2000). Mutations in galectin-1, Sema3A, neuropilin-2, p75NTR, CHL1, or N-CAM-180 (Montag-Sallaz *et al.*, 2002; Puche *et al.*, 1996; Schwarting *et al.*, 2000; Tisay *et al.*, 2000; Treloar *et al.*, 1997; Walz *et al.*, 2002) result in subtle perturbations in the overall pattern of olfactory axon projections; however, the role of these molecules in the precision of glomerular targeting has not been examined.

Netrin-1 expression has also been observed within the olfactory epithelium, and its receptor DCC was detected in cells under the neuroepithelium (Livesey and Hunt, 1997). DCC has also been found in the embryonic rodent olfactory bulb, on the granule cells, and on pioneer axons from output neurons (Gad *et al.*, 1997; Shu *et al.*, 2000). A detailed analysis of the spatio-temporal expression patterns of both Netrin-1 and DCC proteins in the developing rat olfactory system showed that the association of Netrin-1 expression near DCC-expressing olfactory axons is restricted to the initial period of olfactory nerve pathfinding, which suggests that Netrin-1 may play a role in the directed outgrowth of the nascent olfactory axons toward the telencephalon (Astic *et al.*, 2002). Another member of the netrin family, Netrin-4, is localized within the lateral olfactory tract and may be involved in promoting neurite elongation (Koch *et al.*, 2000).

Diffusible factors secreted from the bulb that attract axons of olfactory sensory neurons have been proposed in the past (Ressler *et al.*, 1994; Vassar *et al.*, 1994), and putative guidance molecules in olfactory tissue have been identified (Kafitz and Greer, 1997, 1998; Key and Akeson, 1990; Puche and Key, 1996; Schwarting *et al.*, 2000; St John *et al.*, 2000; Tisay and Key, 1999; Yoshihara *et al.*, 1997), but their direct involvement in axon outgrowth and pathfinding is still unknown. In addition, *in vitro* experiments suggest that a

diffusible signal of unknown identity from the olfactory bulb may also direct receptor axons toward the bulb (Goetze *et al.*, 2002).

As the axons grow toward and into the rostral telencephalon, they encounter a variety of ECM molecules, some of which are associated with both axons and glial cells. These include NCAM, L1, laminin, OCAM (olfactory cell adhesion molecule), chondroitin sulfate proteoglycan (Gong and Shipley, 1996; Miragall and Dermietzel, 1992; Treloar *et al.*, 1996; Yoshihara *et al.*, 1997), and galectin-1 (Crandall *et al.*, 2000).

B. Guidance Cues in the Accessory Olfactory System

In many mammalian species, a second olfactory system, the vomeronasal system, is thought to be specialized in the perception of stimuli related to social and reproductive behaviors (Halpern, 1987; Keverne, 1999). The sensory neurons reside within an oblong-shaped structure, the vomeronasal organ (VNO), situated at the rostral end of the nasal cavity. The vomeronasal receptor neurons are bipolar neurons with a dendrite reaching the surface of the epithelium and an axon projecting through the vomeronasal nerves to the accessory olfactory bulb (AOB). The AOB is an oval structure situated on the dorso-posterior surface of the main olfactory bulb. The incoming vomeronasal nerve axons make synaptic contact with dendrites of AOB neurons in specialized structures called glomeruli (Farbman, 1992). Sensory neurons located in the apical portion of the vomeronasal organ innervate glomeruli restricted to the anterior region of the AOB. In contrast, neurons of the basal region of the VNO project their axons to glomeruli located in the posterior half of the AOB (Jia and Halpern, 1996). The molecular mechanisms that orchestrate the segregation of vomeronasal projections to the anterior and posterior halves of the AOB are just beginning to be understood. Two independent families of vomeronasal receptor genes have been characterized, the V1Rs and V2Rs, which encode seven-transmembrane domain proteins thought to represent the mammalian pheromone receptors (Dulac and Axel, 1995; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997). The V1R and the V2R receptor families are expressed by two spatially segregated populations of VNO sensory neurons, such that neurons lining the apical half of the VNO neuroepithelium co-express V1Rs and the G-protein α-subunit, Gαi2, whereas neurons of the basal half of the VNO are both V2R and Goα positive (Dulac, 2000).

One member of the ephrin family, ephrin-A5, is required for the targeting of V1R-expressing vomeronasal axons to the anterior AOB, presumably through interactions with the Eph receptor EphA6 (Knoll *et al.*, 2001). The targeting of apical vomeronasal axons to the anterior AOB is also

dependent on repulsive forces from the posterior AOB that signal, at least in part, through neuropilin-2 (Npn-2) (Cloutier *et al.*, 2002). Npn-2 is required for the targeting of apical vomeronasal axons to the anterior AOB as well as fasciculation of the vomeronasal nerve and also for separation of the main and accessory olfactory projections during development (Cloutier *et al.*, 2002; Walz *et al.*, 2002).

Secreted semaphorins and slits also participate in establishing the glomerular map in the accessory olfactory system. It is likely that a combination of attractive and repulsive cues in the AOB, and possibly in the vomeronasal sensory neurons themselves, allows for the precise targeting of these sensory afferent projections to either posterior or anterior regions of the AOB. The Slit and Robo families of axon guidance molecules function as cues for basal vomeronasal axons (Knoll *et al.*, 2003). Vomeronasal neurons are repelled by Slit proteins *in vitro*, and Robo2 is expressed in vomeronasal neurons located in the basal region of the VNO (Knoll *et al.*, 2003; Marillat *et al.*, 2002).

Sema3F-Npn-2 signaling is essential for fasciculation of the vomeronasal nerve as it courses past the MOB but is largely dispensable for the targeting of apical vomeronasal axons to the anterior AOB. Moreover, Sema3F is required for the segregation of sensory neuron projections within the main and accessory olfactory systems and for accurate laminar targeting of main olfactory sensory neuron axons (Cloutier *et al.*, 2004). Slit-1 is not required for fasciculation of the vomeronasal nerve but is critical for the targeting of basal vomeronasal sensory neuron axons to the posterior AOB (Cloutier *et al.*, 2004). These results show that two families of secreted repellents play complementary roles in the development of primary sensory neuron projections in the accessory olfactory system.

VIII. Analysis of Axon Growth and Guidance

Several techniques have been used to study axonal guidance. The collagen gel assay, the stripe assay, the pipette assay, and the pump assay are very powerful techniques that have been used to study the effects (attraction, repulsion, or collapse) of different molecules on given axonal populations. In order to study the formation of axon tracts *in vivo,* researchers have used both tract tracing techniques using fluorescent dyes such as DiI or *in vivo* imaging by diffusion tensor magnetic resonance imaging (DTI-MRI). This nondestructive technique can be used to survey the development of multiple axonal tracts in three dimensions in the embryonic, postnatal, and adult brains.

A. The Collagen Gel Assay

The three-dimensional collagen gel assay is one of the most widely used techniques for analyzing axon growth from explants. Collagen is a physiologically relevant biological matrix that allows neurites to grow and soluble factors to diffuse. Generally, two explants or one explant and a source of a putative guidance factor are placed side-by-side in the gel. One explant contains the cells being studied (for example, the dorsal spinal cord containing commissural neurons). As these axons grow into the collagen, they encounter guidance factors released from a second explant (such as the floor plate). The number of axons emanating from the side toward the second, or target, explant can be compared with the number of axons projecting from the side away from the target. The number of axon turning within the gel can also be analyzed. The collagen gel assay was first used by Lumsden and Davies (1983), using co-cultured embryonic mouse sensory neurons, but has since been used to identify and characterize a number of different axonal guidance molecules.

B. The Stripe Assay

In order to elucidate the mechanisms involved in axonal guidance by membrane-bound cues, especially with regard to the topographic targeting of retinal ganglion cell axons within the tectum, Friedrich Bonhoeffer and colleagues developed the "stripe assay" (Walter *et al.*, 1987a,b). In this assay, retinal explants are grown on membrane stripes made from alternating rostral and caudal parts of the tectum. Membrane fragments of two different sources are arranged as a carpet of very narrow alternating strips. Axons growing on such striped carpets are simultaneously confronted with the two substrates at the stripe borders. If there is a preference of axons for one or the other substrate, axons become re-oriented and grow within the lanes of the preferred substrate. Such preferential growth can be due to an increased affinity for attractive factors on the preferred stripes or avoidance of repulsive factors on the alternate stripes, and thus adequate controls must be used to differentiate between these two possibilities. Tectal cell membranes are an excellent substrate for the growth of retinal axons. Results using tissues from developing chicks or rodents show that temporal RGC axons avoid stripes made up of caudal tectal tissue, while nasal RGC axons grow equally well on either rostral or caudal tectal tissue stripes (Godement and Bonhoeffer, 1989; Roskies and O'Leary, 1994; Walter *et al.*, 1987a,b), or show a preference for nasal stripes providing specific pre-treatments (von Boxberg *et al.*, 1993).

These experiments suggest the existence of biochemical labels for the specification of axon–target interactions and provide the conceptual basis for the work of Friedrich Bonhoeffer and others that ultimately led to the identification of specific molecular guidance factors for retinotectal topography.

C. The Pipette Assay

Originally pioneered by Mu-Ming Poo and colleagues, the "*Xenopus* growth cone turning assay" has recently become a popular assay for assessing growth cone turning responses to gradients of guidance factors. The turning assay uses cultured *Xenopus* spinal neurons to examine the cytoplasmic events associated with neurite growth and the response of the growth cone toward extracellular guidance cues. This method uses repetitive pulse application to create reproducible chemical gradients. Microscopic gradients are generated by repetitive pulsatile ejection of picoliters of chemical solution near the growth cone. The gradient generated is a stable gradient over a distance of tens of microns within several minutes. Growth cones of isolated *Xenopus* spinal neurons in culture exhibit chemotactic turning responses when exposed to a gradient of Netrin-1 (Ming *et al.*, 2002). The gradient is produced by repetitive pulsatile ejection of picoliters of solution containing Netrin-1 from a micropipette, positioned at a 45° angle with respect to the direction of initial neurite extension and a distance of 100 μm away from the center of the growth cone. By applying defined extracellular gradients of guidance molecules, early responses of the growth cone to specific guidance cues can be examined as well as the involvement of various cytoplasmic signaling pathways in regulating the turning decision of the growth cone.

Using this assay, Poo and colleagues had previously demonstrated that growth cones from young *Xenopus* retinal explants extend toward a gradient of Netrin-1 protein and away from a gradient of Sema3A (de la Torre *et al.*, 1997; Song *et al.*, 1998). In addition to inducing transient collapse and branching, Sema3A can act as a directional guidance cue in the growth cone turning assay for retinal growth cones (Campbell *et al.*, 2001).

D. The Pump Assay

Geoffrey Goodhill and colleagues designed a technique for generating precise and reproducible gradients of diffusible molecules in collagen gels (Narasimhan, 2004; Rosoff *et al.*, 2004). The assay establishes molecular gradients by printing drops of solution onto the surface of a thin collagen gel. Based on the mechanics of diffusion, the shape and steepness of the

gradient can be controlled, and the actual concentration of molecules produced by this method can be measured quantitatively with fluorescence imaging. Using this technology, they showed that growth cones are capable of detecting a concentration difference as small as about one molecule across their spatial extent. Furthermore, this sensitivity exists across only a relatively small range of ligand concentrations, indicating that adaptation in these growth cones is limited. Their gradient generation method allows for the production of large numbers of identical gradients that require only limited quantities of chemotropic molecules. Moreover, the gradients are stable for at least a day or two after they have been generated. This technique could also be used to generate gradients of multiple factors with different shapes and arbitrary spatial relationships. This powerful new technology can be applied to quantitative studies of other biological processes controlled by molecular gradients, such as cell migration, as well axonal regeneration following injury.

E. The Organotypic Slice Assay

In mammals, surgical manipulation is difficult before birth. Thus, many researchers have turned to using slice preparations to study axonal growth and guidance. Slices maintained in stationary culture with the interface method are ideally suited for manipulations that demonstrate the guidance properties of a prospective target tissue (Gahwiler *et al.*, 1997). For example, to better understand the projection of the commissural axons in the spinal cord, an "open book" organotypic slice assay was used, in which sections of spinal cord are split at the midline, flattened, and cultured in collagen to analyze the trajectories of the commissural axons. Advances in live optical imaging, including confocal and two-photon microscopy using GFP-based markers, allow the direct observation of axonal growth within the slice. Thus, using this assay, live growing axons can be studied *in situ* with maximum resolution and precision.

F. MRI/DTI

Magnetic resonance imaging (MRI) uses a strong electromagnetic field to align hydrogen atoms in the body in parallel with the magnetic field, in either the same or the opposite direction of the field. Hydrogen atoms become excited and resonate with the exciting wave. As the hydrogen atoms return to their original energy state, energy is released in the form of radio waves, which are detected by the MRI machine. Diffusion tensor imaging (DTI) measures the alignment of water molecules in the brain. Water molecules

preferentially align within ordered structures in the brain such as axonal tract. The vector orientation of their alignment can be observed and color-coded to give a visual image of the position and direction of axon tracts within the brain. DTI can be combined with conventional MRI to capture T1- and T2-weighted images such that a three-dimensional map of the brain can be generated (Zhang *et al.*, 2003).

Recent advances in magnetic resonance research have opened up new opportunities for gathering functional information about the brain. The development of DTI has offered the possibility to go beyond anatomical imaging to study tissue structure at a microscopic level *in vivo*. Currently, DTI provides an invaluable tool for the study and diagnosis of white-matter diseases. An important application of DTI is called "fiber tracking," which follows the vector trajectories from given coordinates with the brain to "trace" axonal pathways. Combined with fMRI, information about white-matter tracts reveals important information about neurocognitive networks and may improve our understanding of brain function. Future advances could involve the use of magnetically labeled molecules to analyze axons expressing specific molecules *in vivo*, possibly in living brains.

IX. Conclusions

The establishment of correct neuronal connections is crucial for proper functioning of the vertebrate nervous system. Such precise wiring of neuronal connections is also required in the adult brain for functional recovery after brain injury and disease. To reach their proper targets, axons rely upon the expression of highly conserved families of attractive and repulsive guidance molecules, including the Netrins, Slits, semaphorins, and ephrins. These guidance systems are used to generate an astonishingly varied set of neuronal circuits. It is difficult to understand how such complexity in brain wiring is derived from such a relatively small set of molecules. The regulation of guidance receptors and ligands allows a single guidance system to generate a variety of different responses, but even these cannot account for the huge number of different connections made in the nervous system. What is clear is that the same molecules are used over and over in establishing different projections within the brain and spinal cord. Furthermore, although they may function in slightly different ways, the molecules themselves are conserved from flies and worms to humans. Recent evidence suggests that additional layers of complexity may exist where molecules and receptors from different molecular families interact in signaling to create more complicated responses. We do not know exactly how growth cones are able to respond to molecular gradients within the environment, nor exactly how these signals are relayed intracellularly. Furthermore, little is known about

how axons are able to respond to multiple gradients simultaneously. Because neuronal regeneration is, to a certain extent, a recapitulation of development, understanding the mechanisms regulating axon guidance will not only help delineate the pathoetiology of various neurological disorders, due to erroneous axon pathfinding, but also shed light on potential ways of clinically repairing the injured nervous system.

References

Ahmad, F. J., Pienkowski, T. P., and Baas, P. W. (1993). Regional differences in microtubule dynamics in the axon. *J. Neurosci.* **13,** 856–866.

Aizawa, H., Wakatsuki, S., Ishii, A., Moriyama, K., Sasaki, Y., Ohashi, K., Sekine-Aizawa, Y., Sehara-Fujisawa, A., Mizuno, K., Goshima, Y., and Yahara, I. (2001). Phosphorylation of cofilin by LIM-kinase is necessary for semaphorin 3A-induced growth cone collapse. *Nat. Neurosci.* **4,** 367–373.

Alcantara, S., Ruiz, M., De Castro, F., Soriano, E., and Sotelo, C. (2000). Netrin 1 acts as an attractive or as a repulsive cue for distinct migrating neurons during the development of the cerebellar system. *Development* **127,** 1359–1372.

Altman, J., and Bayer, S. A. (1984). The development of the rat spinal cord. *Adv Anat. Embryol. Cell Biol.* **85,** 1–164.

Alvarez-Bolado, G., Schwarz, M., and Gruss, P. (1997). Pax-2 in the chiasm. *Cell Tissue Res.* **290,** 197–200.

Anderson, R. B., and Holt, C. E. (2002). Expression of UNC-5 in the developing Xenopus visual system. *Mech. Dev.* **118,** 157–160.

Anderson, R. B., Walz, A., Holt, C. E., and Key, B. (1998). Chondroitin sulfates modulate axon guidance in embryonic Xenopus brain. *Dev. Biol.* **202,** 235–243.

Astic, L., Pellier-Monnin, V., Saucier, D., Charrier, C., and Mehlen, P. (2002). Expression of netrin-1 and netrin-1 receptor, DCC, in the rat olfactory nerve pathway during development and axonal regeneration. *Neuroscience* **109,** 643–656.

Attardi, D. G., and Sperry, R. W. (1963). Preferential selection of central pathways by regenerating optic fibers. *Exp. Neurol.* **7,** 46–64.

Augsburger, A., Schuchardt, A., Hoskins, S., Dodd, J., and Butler, S. (1999). BMPs as mediators of roof plate repulsion of commissural neurons. *Neuron* **24,** 127–141.

Auld, V. (1999). Glia as mediators of growth cone guidance: Studies from insect nervous systems. *Cell Mol. Life Sci.* **55,** 1377–1385.

Bagri, A., Marin, O., Plump, A. S., Mak, J., Pleasure, S. J., Rubenstein, J. L., and Tessier-Lavigne, M. (2002). Slit proteins prevent midline crossing and determine the dorsoventral position of major axonal pathways in the mammalian forebrain. *Neuron* **33,** 233–248.

Bak, M., and Fraser, S. E. (2003). Axon fasciculation and differences in midline kinetics between pioneer and follower axons within commissural fascicles. *Development* **130,** 4999–5008.

Bamburg, J. R. (2003). Introduction to cytoskeletal dynamics and pathfinding of neuronal growth cones. *J. Histochem. Cytochem.* **51,** 407–409.

Banyai, L., and Patthy, L. (1999). The NTR module: Domains of netrins, secreted frizzled related proteins, and type I procollagen C-proteinase enhancer protein are homologous with tissue inhibitors of metalloproteases. *Protein Sci.* **8,** 1636–1642.

Barbieri, A. M., Broccoli, V., Bovolenta, P., Alfano, G., Marchitiello, A., Mocchetti, C., Crippa, L., Bulfone, A., Marigo, V., Ballabio, A., and Banfi, S. (2002). Vax2 inactivation in

mouse determines alteration of the eye dorsal-ventral axis, misrouting of the optic fibres and eye coloboma. *Development* **129,** 805–813.

Bartsch, U., Kirchhoff, F., and Schachner, M. (1989). Immunohistological localization of the adhesion molecules L1, N-CAM, and MAG in the developing and adult optic nerve of mice. *J. Comp. Neurol.* **284,** 451–462.

Bastiani, M. J., Raper, J. A., and Goodman, C. S. (1984). Pathfinding by neuronal growth cones in grasshopper embryos. III. Selective affinity of the G growth cone for the P cells within the A/P fascicle. *J. Neurosci.* **4,** 2311–2328.

Bastiani, M. J., du Lac, S., and Goodman, C. S. (1986). Guidance of neuronal growth cones in the grasshopper embryo. I. Recognition of a specific axonal pathway by the pCC neuron. *J. Neurosci.* **6,** 3518–3531.

Bate, C. M. (1976). Pioneer neurones in an insect embryo. *Nature* **260,** 54–56.

Baumeister, H., Gronostajski, R. M., Lyons, G. E., and Margolis, F. L. (1999). Identification of NFI-binding sites and cloning of NFI-cDNAs suggest a regulatory role for NFI transcription factors in olfactory neuron gene expression. *Brain Res. Mol. Brain Res.* **72,** 65–79.

Bear, J. E., Svitkina, T. M., Krause, M., Schafer, D. A., Loureiro, J. J., Strasser, G. A., Maly, I. V., Chaga, O. Y., Cooper, J. A., Borisy, G. G., and Gertler, F. B. (2002). Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility. *Cell* **109,** 509–521.

Behrens, M., Venkatraman, G., Gronostajski, R. M., Reed, R. R., and Margolis, F. L. (2000). NFI in the development of the olfactory neuroepithelium and the regulation of olfactory marker protein gene expression. *Eur. J. Neurosci.* **12,** 1372–1384.

Bentley, D., and Caudy, M. (1983). Pioneer axons lose directed growth after selective killing of guidepost cells. *Nature* **304,** 62–65.

Bentley, D., and O'Connor, T. P. (1994). Cytoskeletal events in growth cone steering. *Curr. Opin. Neurobiol.* **4,** 43–48.

Bertuzzi, S., Hindges, R., Mui, S. H., O'Leary, D. D., and Lemke, G. (1999). The homeodomain protein vax1 is required for axon guidance and major tract formation in the developing forebrain. *Genes Dev.* **13,** 3092–3105.

Bhanot, P., Brink, M., Samos, C. H., Hsieh, J. C., Wang, Y., Macke, J. P., Andrew, D., Nathans, J., and Nusse, R. (1996). A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. *Nature* **382,** 225–230.

Birgbauer, E., Cowan, C. A., Sretavan, D. W., and Henkemeyer, M. (2000). Kinase independent function of EphB receptors in retinal axon pathfinding to the optic disc from dorsal but not ventral retina. *Development* **127,** 1231–1241.

Bloch-Gallego, E., Ezan, F., Tessier-Lavigne, M., and Sotelo, C. (1999). Floor plate and netrin-1 are involved in the migration and survival of inferior olivary neurons. *J. Neurosci.* **19,** 4407–4420.

Bovolenta, P., and Dodd, J. (1990). Guidance of commissural growth cones at the floor plate in embryonic rat spinal cord. *Development* **109,** 435–447.

Bovolenta, P., and Fernaud-Espinosa, I. (2000). Nervous system proteoglycans as modulators of neurite outgrowth. *Prog. Neurobiol.* **61,** 113–132.

Braisted, J. E., McLaughlin, T., Wang, H. U., Friedman, G. C., Anderson, D. J., and O'Leary, D, D. (1997). Graded and lamina-specific distributions of ligands of EphB receptor tyrosine kinases in the developing retinotectal system. *Dev. Biol.* **191,** 14–28.

Braisted, J. E., Catalano, S. M., Stimac, R., Kennedy, T. E., Tessier-Lavigne, M., Shatz, C. J., and O'Leary, D. D. (2000). Netrin-1 promotes thalamic axon growth and is required for proper development of the thalamocortical projection. *J. Neurosci.* **20,** 5792–5801.

Bray, D., and Chapman, K. (1985). Analysis of microspike movements on the neuronal growth cone. *J. Neurosci.* **5,** 3204–3213.

Bridgman, P. C., and Dailey, M. E. (1989). The organization of myosin and actin in rapid frozen nerve growth cones. *J. Cell Biol.* **108,** 95–109.

Brittis, P. A., and Silver, J. (1994). Exogenous glycosaminoglycans induce complete inversion of retinal ganglion cell bodies and their axons within the retinal neuroepithelium. *Proc. Natl. Acad. Sci. USA* **91,** 7539–7542.

Brittis, P. A., and Silver, J. (1995). Multiple factors govern intraretinal axon guidance: A time-lapse study. *Mol. Cell Neurosci.* **6,** 413–432.

Brittis, P. A., Lu, Q., and Flanagan, J. G. (2002). Axonal protein synthesis provides a mechanism for localized regulation at an intermediate target. *Cell* **110,** 223–235.

Brose, K., and Tessier-Lavigne, M. (2000). Slit proteins: Key regulators of axon guidance, axonal branching, and cell migration. *Curr. Opin. Neurobiol.* **10,** 95–102.

Brose, K., Bland, K. S., Wang, K. H., Arnott, D., Henzel, W., Goodman, C. S., Tessier-Lavigne, M., and Kidd, T. (1999). Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. *Cell* **96,** 795–806.

Brown, A., Yates, P. A., Burrola, P., Ortuno, D., Vaidya, A., Jessell, T. M., Pfaff, S. L., O'Leary, D. D., and Lemke, G. (2000). Topographic mapping from the retina to the midbrain is controlled by relative but not absolute levels of EphA receptor signaling. *Cell* **102,** 77–88.

Bruckner, K., Pasquale, E. B., and Klein, R. (1997). Tyrosine phosphorylation of transmembrane ligands for Eph receptors. *Science* **275,** 1640–1643.

Brunjes, P. C., and Frazier, L. L. (1986). Maturation and plasticity in the olfactory system of vertebrates. *Brain Res.* **396,** 1–45.

Bulow, H. E., and Hobert, O. (2004). Differential sulfations and epimerization define heparan sulfate specificity in nervous system development. *Neuron* **41,** 723–736.

Butler, S. J., and Dodd, J. (2003). A role for BMP heterodimers in roof plate-mediated repulsion of commissural axons. *Neuron* **38,** 389–401.

Campbell, D. S., Regan, A. G., Lopez, J. S., Tannahill, D., Harris, W. A., and Holt, C. E. (2001). Semaphorin 3A elicits stage-dependent collapse, turning, and branching in Xenopus retinal growth cones. *J. Neurosci.* **21,** 8538–8547.

Carlier, M. F., Laurent, V., Santolini, J., Melki, R., Didry, D., Xia, G. X., Hong, Y., Chua, N. H., and Pantaloni, D. (1997). Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: Implication in actin-based motility. *J. Cell Biol.* **136,** 1307–1322.

Carney, P. R., and Silver, J. (1983). Studies on cell migration and axon guidance in the developing distal auditory system of the mouse. *J. Comp. Neurol.* **215,** 359–369.

Castellani, V., Chedotal, A., Schachner, M., Faivre-Sarrailh, C., and Rougon, G. (2000). Analysis of the L1-deficient mouse phenotype reveals cross-talk between Sema3A and L1 signaling pathways in axonal guidance. *Neuron* **27,** 237–249.

Castellani, V., De Angelis, E., Kenwrick, S., and Rougon, G. (2002). Cis and trans interactions of L1 with neuropilin-1 control axonal responses to semaphorin 3A. *EMBO J.* **21,** 6348–6357.

Chae, T., Kwon, Y. T., Bronson, R., Dikkes, P., Li, E., and Tsai, L. H. (1997). Mice lacking p35, a neuronal specific activator of Cdk5, display cortical lamination defects, seizures, and adult lethality. *Neuron* **18,** 29–42.

Chakraborty, T., Ebel, F., Domann, E., Niebuhr, K., Gerstel, B., Pistor, S., Temm-Grove, C. J., Jockusch, B. M., Reinhard, M., Walter, U., *et al.* (1995). A focal adhesion factor directly linking intracellularly motile Listeria monocytogenes and Listeria ivanovii to the actin-based cytoskeleton of mammalian cells. *EMBO J.* **14,** 1314–1321.

Challacombe, J. F., Snow, D. M., and Letourneau, P. C. (1996). Actin filament bundles are required for microtubule reorientation during growth cone turning to avoid an inhibitory guidance cue. *J. Cell Sci.* **109**(Pt. 8), 2031–2040.

Challacombe, J. F., Snow, D. M., and Letourneau, P. C. (1997). Dynamic microtubule ends are required for growth cone turning to avoid an inhibitory guidance cue. *J. Neurosci.* **17,** 3085–3095.

Charron, F., Stein, E., Jeong, J., McMahon, A. P., and Tessier-Lavigne, M. (2003). The morphogen sonic hedgehog is an axonal chemoattractant that collaborates with netrin-1 in midline axon guidance. *Cell* **113,** 11–23.

Chen, H., Chedotal, A., He, Z., Goodman, C. S., and Tessier-Lavigne, M. (1997). Neuropilin-2, a novel member of the neuropilin family, is a high affinity receptor for the semaphorins Sema E and Sema IV but not Sema III. *Neuron* **19,** 547–559.

Cheng, H. J., Nakamoto, M., Bergemann, A. D., and Flanagan, J. G. (1995). Complementary gradients in expression and binding of ELF-1 and Mek4 in development of the topographic retinotectal projection map. *Cell* **82,** 371–381.

Cheng, H. J., Bagri, A., Yaron, A., Stein, E., Pleasure, S. J., and Tessier-Lavigne, M. (2001). Plexin-A3 mediates semaphorin signaling and regulates the development of hippocampal axonal projections. *Neuron* **32,** 249–263.

Chitnis, A. B., and Kuwada, J. Y. (1990). Axonogenesis in the brain of zebrafish embryos. *J. Neurosci.* **10,** 1892–1905.

Chung, K. Y., Shum, D. K., and Chan, S. O. (2000a). Expression of chondroitin sulfate proteoglycans in the chiasm of mouse embryos. *J. Comp. Neurol.* **417,** 153–63.

Chung, K. Y., Taylor, J. S., Shum, D. K., and Chan, S. O. (2000b). Axon routing at the optic chiasm after enzymatic removal of chondroitin sulfate in mouse embryos. *Development* **127,** 2673–2683.

Chung, K. Y., Leung, K. M., Lin, C. C., Tam, K. C., Hao, Y. L., Taylor, J. S., and Chan, S. O. (2004). Regionally specific expression of L1 and sialylated NCAM in the retinofugal pathway of mouse embryos. *J. Comp. Neurol.* **471,** 482–498.

Cloutier, J. F., Giger, R. J., Koentges, G., Dulac, C., Kolodkin, A. L., and Ginty, D. D. (2002). Neuropilin-2 mediates axonal fasciculation, zonal segregation, but not axonal convergence, of primary accessory olfactory neurons. *Neuron* **33,** 877–892.

Cloutier, J. F., Sahay, A., Chang, E. C., Tessier-Lavigne, M., Dulac, C., Kolodkin, A. L., and Ginty, D. D. (2004). Differential requirements for semaphorin 3F and Slit-1 in axonal targeting, fasciculation, and segregation of olfactory sensory neuron projections. *J. Neurosci.* **24,** 9087–9096.

Cohen, N. R., Taylor, J. S., Scott, L. B., Guillery, R. W., Soriano, P., and Furley, A. J. (1998). Errors in corticospinal axon guidance in mice lacking the neural cell adhesion molecule L1. *Curr. Biol.* **8,** 26–33.

Colamarino, S. A., and Tessier-Lavigne, M. (1995). The role of the floor plate in axon guidance. *Annu. Rev. Neurosci.* **18,** 497–529.

Colavita, A., Krishna, S., Zheng, H., Padgett, R. W., and Culotti, J. G. (1998). Pioneer axon guidance by UNC-129, a C. elegans TGF-beta. *Science* **281,** 706–709.

Comeau, M. R., Johnson, R., Du Bose, R. F., Petersen, M., Gearing, P., Vanden Bos, T., Park, L., Farrah, T., Buller, R. M., Cohen, J. I., Strockbine, L. D., Rauch, C., and Spriggs, M. K. (1998). A poxvirus-encoded semaphorin induces cytokine production from monocytes and binds to a novel cellular semaphorin receptor, VESPR. *Immunity* **8,** 473–482.

Condic, M. L., Snow, D. M., and Letourneau, P. C. (1999). Embryonic neurons adapt to the inhibitory proteoglycan aggrecan by increasing integrin expression. *J. Neurosci.* **19,** 10036–10043.

Cooper, N. G., and Steindler, D. A. (1986). Monoclonal antibody to glial fibrillary acidic protein reveals a parcellation of individual barrels in the early postnatal mouse somatosensory cortex. *Brain Res.* **380,** 341–348.

Couch, J., and Condron, B. (2002). Axon guidance: Comm hither, Robo. *Curr. Biol.* **12,** R741–R742.

Crandall, J. E., Dibble, C., Butler, D., Pays, L., Ahmad, N., Kostek, C., Puschel, A. W., and Schwarting, G. A. (2000). Patterning of olfactory sensory connections is mediated by extracellular matrix proteins in the nerve layer of the olfactory bulb. *J. Neurobiol.* **45,** 195–206.

Croucher, S. J., and Tickle, C. (1989). Characterization of epithelial domains in the nasal passages of chick embryos: Spatial and temporal mapping of a range of extracellular matrix and cell surface molecules during development of the nasal placode. *Development* **106,** 493–509.

Cucchiaro, J. B. (1991). Early development of the retinal line of decussation in normal and albino ferrets. *J. Comp. Neurol.* **312,** 193–206.

Cummings, D. M., Malun, D., and Brunjes, P. C. (1997). Development of the anterior commissure in the opossum: Midline extracellular space and glia coincide with early axon decussation. *J. Neurobiol.* **32,** 403–414.

Cutforth, T., and Harrison, C. J. (2002). Ephs and ephrins close ranks. *Trends Neurosci.* **25,** 332–334.

Cutforth, T., Moring, L., Mendelsohn, M., Nemes, A., Shah, N. M., Kim, M. M., Frisen, J., and Axel, R. (2003). Axonal ephrin-As and odorant receptors: Coordinate determination of the olfactory sensory map. *Cell* **114,** 311–322.

Dahme, M., Bartsch, U., Martini, R., Anliker, B., Schachner, M., and Mantei, N. (1997). Disruption of the mouse L1 gene leads to malformations of the nervous system. *Nat. Genet.* **17,** 346–349.

das Neves, L., Duchala, C. S., Tolentino-Silva, F., Haxhiu, M. A., Colmenares, C., Macklin, W. B., Campbell, C. E., Butz, K. G., and Gronostajski, R. M. (1999). Disruption of the murine nuclear factor I-A gene (Nfia) results in perinatal lethality, hydrocephalus, and agenesis of the corpus callosum. *Proc. Natl. Acad. Sci. USA* **96,** 11946–11951.

Davis, J. Q., and Bennett, V. (1994). Ankyrin binding activity shared by the neurofascin/L1/NrCAM family of nervous system cell adhesion molecules. *J. Biol. Chem* **269,** 27163–27166.

De Carlos, J. A., and O'Leary, D. D. (1992). Growth and targeting of subplate axons and establishment of major cortical pathways. *J. Neurosci.* **12,** 1194–1211.

de la Torre, J. R., Hopker, V. H., Ming, G. L., Poo, M. M., Tessier-Lavigne, M., Hemmati-Brivanlou, A., and Holt, C. E. (1997). Turning of retinal growth cones in a netrin-1 gradient mediated by the netrin receptor DCC. *Neuron* **19,** 1211–1224.

de Wit, J., and Verhaagen, J. (2003). Role of semaphorins in the adult nervous system. *Prog. Neurobiol.* **71,** 249–267.

Deiner, M. S., and Sretavan, D. W. (1999). Altered midline axon pathways and ectopic neurons in the developing hypothalamus of netrin-1- and DCC-deficient mice. *J. Neurosci.* **19,** 9900–9912.

Deiner, M. S., Kennedy, T. E., Fazeli, A., Serafini, T., Tessier-Lavigne, M., and Sretavan, D. W. (1997). Netrin-1 and DCC mediate axon guidance locally at the optic disc: Loss of function leads to optic nerve hypoplasia. *Neuron* **19,** 575–589.

Del Rio, J. A., Heimrich, B., Borrell, V., Forster, E., Drakew, A., Alcantara, S., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K., Derer, P., Frotscher, M., and Soriano, E. (1997). A role for Cajal-Retzius cells and reelin in the development of hippocampal connections. *Nature* **385,** 70–74.

Del Rio, J. A., Gonzalez-Billault, C., Urena, J. M., Jimenez, E. M., Barallobre, M. J., Pascual, M., Pujadas, L., Simo, S., La Torre, A., Wandosell, F., Avila, J., and Soriano, E. (2004). MAP1B is required for Netrin 1 signaling in neuronal migration and axonal guidance. *Curr. Biol* **14,** 840–850.

Demyanenko, G. P., and Maness, P. F. (2003). The L1 cell adhesion molecule is essential for topographic mapping of retinal axons. *J. Neurosci.* **23,** 530–538.

Demyanenko, G. P., Tsai, A. Y., and Maness, P. F. (1999). Abnormalities in neuronal process extension, hippocampal development, and the ventricular system of L1 knockout mice. *J. Neurosci.* **19,** 4907–4920.

Demyanenko, G. P., Shibata, Y., and Maness, P. F. (2001). Altered distribution of dopaminergic neurons in the brain of L1 null mice. *Brain Res. Dev. Brain Res.* **126,** 21–30.

Dent, E. W., and Gertler, F. B. (2003). Cytoskeletal dynamics and transport in growth cone motility and axon guidance. *Neuron* **40,** 209–227.

Dent, E. W., and Kalil, K. (2001). Axon branching requires interactions between dynamic microtubules and actin filaments. *J. Neurosci.* **21,** 9757–69.

Dent, E. W., Callaway, J. L., Szebenyi, G., Baas, P. W., and Kalil, K. (1999). Reorganization and movement of microtubules in axonal growth cones and developing interstitial branches. *J. Neurosci.* **19,** 8894–8908.

Dent, E. W., Barnes, A. M., Tang, F., and Kalil, K. (2004). Netrin-1 and semaphorin 3A promote or inhibit cortical axon branching, respectively, by reorganization of the cytoskeleton. *J. Neurosci.* **24,** 3002–3012.

Dickson, B. J. (2001). Rho GTPases in growth cone guidance. *Curr. Opin. Neurobiol.* **11,** 103–110.

Diefenbach, T. J., Guthrie, P. B., and Kater, S. B. (2000). Stimulus history alters behavioral responses of neuronal growth cones. *J. Neurosci.* **20,** 1484–1494.

Dodd, J., Morton, S. B., Karagogeos, D., Yamamoto, M., and Jessell, T. M. (1988). Spatial regulation of axonal glycoprotein expression on subsets of embryonic spinal neurons. *Neuron* **1,** 105–116.

Doherty, P., Barton, C. H., Dickson, G., Seaton, P., Rowett, L. H., Moore, S. E., Gower, H. J., and Walsh, F. S. (1989). Neuronal process outgrowth of human sensory neurons on monolayers of cells transfected with cDNAs for five human N-CAM isoforms. *J. Cell Biol.* **109,** 789–798.

Doherty, P., Cohen, J., and Walsh, F. S. (1990). Neurite outgrowth in response to transfected N-CAM changes during development and is modulated by polysialic acid. *Neuron* **5,** 209–219.

Dou, C. L., and Levine, J. M. (1994). Inhibition of neurite growth by the NG2 chondroitin sulfate proteoglycan. *J. Neurosci.* **14,** 7616–7628.

Drescher, U., Bonhoeffer, F., and Muller, B. K. (1997). The Eph family in retinal axon guidance. *Curr. Opin. Neurobiol.* **7,** 75–80.

du Lac, S., Bastiani, M. J., and Goodman, C. S. (1986). Guidance of neuronal growth cones in the grasshopper embryo. II. Recognition of a specific axonal pathway by the aCC neuron. *J. Neurosci.* **6,** 3532–3541.

Dulac, C. (2000). Sensory coding of pheromone signals in mammals. *Curr. Opin. Neurobiol.* **10,** 511–518.

Dulac, C., and Axel, R. (1995). A novel family of genes encoding putative pheromone receptors in mammals. *Cell* **83,** 195–206.

Easter, S. S., Jr., Ross, L. S., and Frankfurter, A. (1993). Initial tract formation in the mouse brain. *J. Neurosci.* **13,** 285–299.

Edelman, G. M., and Crossin, K. L. (1991). Cell adhesion molecules: Implications for a molecular histology. *Annu. Rev. Biochem.* **60,** 155–190.

Eickholt, B. J., Walsh, F. S., and Doherty, P. (2002). An inactive pool of GSK-3 at the leading edge of growth cones is implicated in Semaphorin 3A signaling. *J. Cell Biol.* **157,** 211–217.

Emerling, D. E., and Lander, A. D. (1996). Inhibitors and promoters of thalamic neuron adhesion and outgrowth in embryonic neocortex: Functional association with chondroitin sulfate. *Neuron* **17,** 1089–1100.

Erskine, L., Williams, S. E., Brose, K., Kidd, T., Rachel, R. A., Goodman, C. S., Tessier-Lavigne, M., and Mason, C. A. (2000). Retinal ganglion cell axon guidance in the mouse optic chiasm: Expression and function of robos and slits. *J. Neurosci.* **20,** 4975–4982.

Fan, J., Mansfield, S. G., Redmond, T., Gordon-Weeks, P. R., and Raper, J. A. (1993). The organization of F-actin and microtubules in growth cones exposed to a brain-derived collapsing factor. *J. Cell Biol.* **121,** 867–878.

Farbman, A. I. (1992). "Cell Biology of Olfaction." Cambridge University Press, Cambridge, UK.

Fazeli, A., Dickinson, S. L., Hermiston, M. L., Tighe, R. V., Steen, R. G., Small, C. G., Stoeckli, E. T., Keino-Masu, K., Masu, M., Rayburn, H., Simons, J., Bronson, R. T., Gordon, J. I., Tessier-Lavigne, M., and Weinberg, R. A. (1997). Phenotype of mice lacking functional Deleted in colorectal cancer (Dcc) gene. *Nature* **386,** 796–804.

Feldheim, D. A., Kim, Y. I., Bergemann, A. D., Frisen, J., Barbacid, M., and Flanagan, J. G. (2000). Genetic analysis of ephrin-A2 and ephrin-A5 shows their requirement in multiple aspects of retinocollicular mapping. *Neuron* **25,** 563–574.

Fitch, M. T., and Silver, J. (1997). Glial cell extracellular matrix: Boundaries for axon growth in development and regeneration. *Cell Tissue Res.* **290,** 379–384.

Flanagan, J. G., and Vanderhaeghen, P. (1998). The ephrins and Eph receptors in neural development. *Annu. Rev. Neurosci.* **21,** 309–345.

Forscher, P., and Smith, S. J. (1988). Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. *J. Cell Biol.* **107,** 1505–1516.

Fournier, A. E., Nakamura, F., Kawamoto, S., Goshima, Y., Kalb, R. G., and Strittmatter, S. M. (2000). Semaphorin3A enhances endocytosis at sites of receptor-F-actin colocalization during growth cone collapse. *J. Cell Biol.* **149,** 411–422.

Fransen, E., D'Hooge, R., Van Camp, G., Verhoye, M., Sijbers, J., Reyniers, E., Soriano, P., Kamiguchi, H., Willemsen, R., Koekkoek, S. K., De Zeeuw, C. I., De Deyn, P. P., Van der Linden, A., Lemmon, V., Kooy, R. F., and Willems, P. J. (1998). L1 knockout mice show dilated ventricles, vermis hypoplasia and impaired exploration patterns. *Hum. Mol. Genet.* **7,** 999–1009.

Fricke, C., Lee, J. S., Geiger-Rudolph, S., Bonhoeffer, F., and Chien, C. B. (2001). astray, a zebrafish roundabout homolog required for retinal axon guidance. *Science* **292,** 507–510.

Fujisawa, H., and Kitsukawa, T. (1998). Receptors for collapsin/semaphorins. *Curr. Opin. Neurobiol.* **8,** 587–592.

Gad, J. M., Keeling, S. L., Wilks, A. F., Tan, S. S., and Cooper, H. M. (1997). The expression patterns of guidance receptors, DCC and Neogenin, are spatially and temporally distinct throughout mouse embryogenesis. *Dev. Biol.* **192,** 258–273.

Gahwiler, B. H., Capogna, M., Debanne, D., McKinney, R. A., and Thompson, S. M. (1997). Organotypic slice cultures: A technique has come of age. *Trends Neurosci.* **20,** 471–477.

Garcia-Abreu, J., Moura Neto, V., Carvalho, S. L., and Cavalcante, L. A. (1995). Regionally specific properties of midbrain glia: I. Interactions with midbrain neurons. *J. Neurosci. Res.* **40,** 471–477.

Garrity, P. A. (2003). Developmental biology: How neurons avoid derailment. *Nature* **422,** 570–571.

Gazzaniga, M. S. (1995). Principles of human brain organization derived from split-brain studies. *Neuron* **14,** 217–228.

Georgiou, M., and Tear, G. (2002). Commissureless is required both in commissural neurones and midline cells for axon guidance across the midline. *Development* **129,** 2947–2956.

Gerstel, B., Grobe, L., Pistor, S., Chakraborty, T., and Wehland, J. (1996). The ActA polypeptides of Listeria ivanovii and Listeria monocytogenes harbor related binding sites for host microfilament proteins. *Infect. Immun.* **64,** 1929–1936.

Gertler, F. B., Niebuhr, K., Reinhard, M., Wehland, J., and Soriano, P. (1996). Mena, a relative of VASP and Drosophila Enabled, is implicated in the control of microfilament dynamics. *Cell* **87,** 227–239.

Ghosh, A., and Shatz, C. J. (1992). Pathfinding and target selection by developing geniculocortical axons. *J. Neurosci.* **12,** 39–55.

Ghosh, A., and Shatz, C. J. (1993). A role for subplate neurons in the patterning of connections from thalamus to neocortex. *Development* **117,** 1031–1047.

Ghosh, A., Antonini, A., McConnell, S. K., and Shatz, C. J. (1990). Requirement for subplate neurons in the formation of thalamocortical connections. *Nature* **347,** 179–181.

Giger, R. J., Urquhart, E. R., Gillespie, S. K., Levengood, D. V., Ginty, D. D., and Kolodkin, A. L. (1998). Neuropilin-2 is a receptor for semaphorin IV: Insight into the structural basis of receptor function and specificity. *Neuron* **21,** 1079–1092.

Godement, P. (1994). Specific guidance and modulation of growth cone motility during *in vivo* development. *J. Physiol. Paris* **88,** 259–264.

Godement, P., and Bonhoeffer, F. (1989). Cross-species recognition of tectal cues by retinal fibers *in vitro*. *Development* **106,** 313–320.

Goetze, B., Breer, H., and Strotmann, J. (2002). A long-term culture system for olfactory explants with intrinsically fluorescent cell populations. *Chem. Senses* **27,** 817–824.

Goh, K. L., Cai, L., Cepko, C. L., and Gertler, F. B. (2002). Ena/VASP proteins regulate cortical neuronal positioning. *Curr. Biol.* **12,** 565–569.

Goldberg, D. J., and Burmeister, D. W. (1986). Stages in axon formation: Observations of growth of Aplysia axons in culture using video-enhanced contrast-differential interference contrast microscopy. *J. Cell Biol.* **103,** 1921–1931.

Goldberg, D. J., and Burmeister, D. W. (1989). Looking into growth cones. *Trends Neurosci.* **12,** 503–506.

Golding, J. P., Tidcombe, H., Tsoni, S., and Gassmann, M. (1999). Chondroitin sulphate-binding molecules may pattern central projections of sensory axons within the cranial mesenchyme of the developing mouse. *Dev. Biol.* **216,** 85–97.

Gong, Q., and Shipley, M. T. (1996). Expression of extracellular matrix molecules and cell surface molecules in the olfactory nerve pathway during early development. *J. Comp. Neurol.* **366,** 1–14.

Goodman, C. S., Bastiani, M. J., Doe, C. Q., du Lac, S., Helfand, S. L., Kuwada, J. Y., and Thomas, J. B. (1984). Cell recognition during neuronal development. *Science* **225,** 1271–1279.

Gordon-Weeks, P. R. (1991). Microtubule organization in growth cones. *Biochem. Soc. Trans.* **19,** 1080–1085.

Goshima, Y., Nakamura, F., Strittmatter, P., and Strittmatter, S. M. (1995). Collapsin-induced growth cone collapse mediated by an intracellular protein related to UNC-33. *Nature* **376,** 509–514.

Graziadei, P. P. C., and Monti Graziadei, G. A. (1978). The olfactory system: A model for the study of neurogenesis and axon regeneration in mammals. *In* "Neuronal Plasticity" (C. W. Cotman, Ed.), pp. 131–153. Raven Press, New York.

Greferath, U., Canty, A. J., Messenger, J., and Murphy, M. (2002). Developmental expression of EphA4-tyrosine kinase receptor in the mouse brain and spinal cord. *Gene Expr. Patterns* **2,** 267–274.

Grenningloh, G., Soehrman, S., Bondallaz, P., Ruchti, E., and Cadas, H. (2004). Role of the microtubule destabilizing proteins SCG10 and stathmin in neuronal growth. *J. Neurobiol.* **58,** 60–69.

Gronostajski, R. M., Adhya, S., Nagata, K., Guggenheimer, R. A., and Hurwitz, J. (1985). Site-specific DNA binding of nuclear factor I: Analyses of cellular binding sites. *Mol. Cell. Biol.* **5,** 964–971.

Gu, C., Rodriguez, E. R., Reimert, D. V., Shu, T., Fritzsch, B., Richards, L. J., Kolodkin, A. L., and Ginty, D. D. (2003). Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. *Dev. Cell* **5,** 45–57.

Guillery, R. W., Mason, C. A., and Taylor, J. S. (1995). Developmental determinants at the mammalian optic chiasm. *J. Neurosci.* **15,** 4727–4737.

Gundersen, R. W., and Barrett, J. N. (1979). Neuronal chemotaxis: Chick dorsal-root axons turn toward high concentrations of nerve growth factor. *Science* **206,** 1079–1080.

Hall, A. (1998). Rho GTPases and the actin cytoskeleton. *Science* **279,** 509–514.

Hall, A. C., Lucas, F. R., and Salinas, P. C. (2000). Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. *Cell* **100,** 525–535.

Halpern, M. (1987). The organization and function of the vomeronasal system. *Annu. Rev. Neurosci.* **10,** 325–362.

Hamasaki, T., Goto, S., Nishikawa, S., and Ushio, Y. (2001). A role of netrin-1 in the formation of the subcortical structure striatum: Repulsive action on the migration of late-born striatal neurons. *J. Neurosci.* **21,** 4272–4280.

Harris, W. A., Holt, C. E., and Bonhoeffer, F. (1987). Retinal axons with and without their somata, growing to and arborizing in the tectum of Xenopus embryos: A time-lapse video study of single fibres *in vivo*. *Development* **101,** 123–133.

He, Z., and Tessier-Lavigne, M. (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. *Cell* **90,** 739–751.

Hedgecock, E. M., Culotti, J. G., and Hall, D. H. (1990). The unc-5, unc-6, and unc-40 genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in C. elegans. *Neuron* **4,** 61–85.

Henkemeyer, M., Orioli, D., Henderson, J. T., Saxton, T. M., Roder, J., Pawson, T., and Klein, R. (1996). Nuk controls pathfinding of commissural axons in the mammalian central nervous system. *Cell* **86,** 35–46.

Hennighausen, L., Siebenlist, U., Danner, D., Leder, P., Rawlins, D., Rosenfeld, P., and Kelly, T., Jr. (1985). High-affinity binding site for a specific nuclear protein in the human IgM gene. *Nature* **314,** 289–292.

Herrada, G., and Dulac, C. (1997). A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. *Cell* **90,** 763–773.

Herrera, E., Brown, L., Aruga, J., Rachel, R. A., Dolen, G., Mikoshiba, K., Brown, S., and Mason, C. A. (2003). Zic2 patterns binocular vision by specifying the uncrossed retinal projection. *Cell* **114,** 545–557.

Herrera, E., Marcus, R., Li, S., Williams, S. E., Erskine, L., Lai, E., and Mason, C. (2004). Foxd1 is required for proper formation of the optic chiasm. *Development* **131,** 5727–5739.

Hidalgo, A. (2003). Neuron-glia interactions during axon guidance in Drosophila. *Biochem. Soc. Trans.* **31,** 50–55.

Hidalgo, A., and Booth, G. E. (2000). Glia dictate pioneer axon trajectories in the Drosophila embryonic CNS. *Development* **127,** 393–402.

Hidalgo, A., and Brand, A. H. (1997). Targeted neuronal ablation: The role of pioneer neurons in guidance and fasciculation in the CNS of Drosophila. *Development* **124,** 3253–3262.

Hindges, R., McLaughlin, T., Genoud, N., Henkemeyer, M., and O'Leary, D. D. (2002). EphB forward signaling controls directional branch extension and arborization required for dorsal-ventral retinotopic mapping. *Neuron* **35,** 475–487.

Hjorth, J., and Key, B. (2002). Development of axon pathways in the zebrafish central nervous system. *Int. J. Dev. Biol.* **46,** 609–619.

Holland, S. J., Gale, N. W., Mbamalu, G., Yancopoulos, G. D., Henkemeyer, M., and Pawson, T. (1996). Bidirectional signalling through the EPH-family receptor Nuk and its transmembrane ligands. *Nature* **383,** 722–725.

Holley, J. A. (1982). Early development of the circumferential axonal pathway in mouse and chick spinal cord. *J. Comp. Neurol.* **205,** 371–382.

Holmes, G. P., Negus, K., Burridge, L., Raman, S., Algar, E., Yamada, T., and Little, M. H. (1998). Distinct but overlapping expression patterns of two vertebrate slit homologs implies functional roles in CNS development and organogenesis. *Mech. Dev.* **79,** 57–72.

Holt, C. E., and Harris, W. A. (1993). Position, guidance, and mapping in the developing visual system. *J. Neurobiol.* **24,** 1400–1422.

Hopker, V. H., Shewan, D., Tessier-Lavigne, M., Poo, M., and Holt, C. (1999). Growth-cone attraction to netrin-1 is converted to repulsion by laminin-1. *Nature* **401,** 69–73.

Hortsch, M. (1996). The L1 family of neural cell adhesion molecules: Old proteins performing new tricks. *Neuron* **17,** 587–593.

Hu, H. (2001). Cell-surface heparan sulfate is involved in the repulsive guidance activities of Slit2 protein. *Nat. Neurosci.* **4,** 695–701.

Hu, H., Li, M., Labrador, J. P., McEwen, J., Lai, E. C., Goodman, C. S., and Bashaw, G. J. (2005). Cross GTPase-activating protein (CrossGAP)/Vilse links the Roundabout receptor to Rac to regulate midline repulsion. *Proc. Natl. Acad. Sci. USA.*.

Hu, Z., Yue, X., Shi, G., Yue, Y., Crockett, D. P., Blair-Flynn, J., Reuhl, K., Tessarollo, L., and Zhou, R. (2003). Corpus callosum deficiency in transgenic mice expressing a truncated ephrin-A receptor. *J. Neurosci.* **23,** 10963–10970.

Huot, J. (2004). Ephrin signaling in axon guidance. *Prog. Neuropsychopharmacol. Biol. Psychiat.* **28,** 813–818.

Hutson, L. D., and Chien, C. B. (2002). Pathfinding and error correction by retinal axons: The role of astray/robo2. *Neuron* **33,** 205–217.

Hutton, L. A., Gu, G., and Simerly, R. B. (1998). Development of a sexually dimorphic projection from the bed nuclei of the stria terminalis to the anteroventral periventricular nucleus in the rat. *J. Neurosci.* **18,** 3003–3013.

Hynes, R. O. (2002). Integrins: Bidirectional, allosteric signaling machines. *Cell* **110,** 673–687.

Ibanez, M. A., Gu, G., and Simerly, R. B. (2001). Target-dependent sexual differentiation of a limbic-hypothalamic neural pathway. *J. Neurosci.* **21,** 5652–5659.

Imondi, R., and Kaprielian, Z. (2001). Commissural axon pathfinding on the contralateral side of the floor plate: A role for B-class ephrins in specifying the dorsoventral position of longitudinally projecting commissural axons. *Development* **128,** 4859–4871.

Imondi, R., Wideman, C., and Kaprielian, Z. (2000). Complementary expression of transmembrane ephrins and their receptors in the mouse spinal cord: A possible role in constraining the orientation of longitudinally projecting axons. *Development* **127,** 1397–1410.

Inatani, M., Irie, F., Plump, A. S., Tessier-Lavigne, M., and Yamaguchi, Y. (2003). Mammalian brain morphogenesis and midline axon guidance require heparan sulfate. *Science* **302,** 1044–1046.

Ingham, P. W., and McMahon, A. P. (2001). Hedgehog signaling in animal development: Paradigms and principles. *Genes Dev.* **15,** 3059–3087.

Innocenti, G. M. (1986). General organization of callosal connections in the cerebral cortex. *In* "Cerebral Cortex" (E. G. Jones and A. Peters, Eds.), Vol. 5, pp. 291–353. Plenum Press, New York.

Irie, A., Yates, E. A., Turnbull, J. E., and Holt, C. E. (2002). Specific heparan sulfate structures involved in retinal axon targeting. *Development* **129,** 61–70.

Ishii, N., Wadsworth, W. G., Stern, B. D., Culotti, J. G., and Hedgecock, E. M. (1992). UNC-6, a laminin-related protein, guides cell and pioneer axon migrations in C. elegans. *Neuron* **9,** 873–881.

Itoh, A., Miyabayashi, T., Ohno, M., and Sakano, S. (1998). Cloning and expressions of three mammalian homologues of Drosophila slit suggest possible roles for Slit in the formation and maintenance of the nervous system. *Brain Res. Mol. Brain Res.* **62,** 175–186.

Jacobs, J. R., and Goodman, C. S. (1989). Embryonic development of axon pathways in the Drosophila CNS. I. A glial scaffold appears before the first growth cones. *J. Neurosci.* **9,** 2402–2411.

Jenzora, A., Behrendt, B., Small, J. V., Wehland, J., and Stradal, T. E. (2005). PREL1 provides a link from Ras signalling to the actin cytoskeleton via Ena/VASP proteins. *FEBS Lett.* **579,** 455–463.

Jessell, T. M. (1988). Adhesion molecules and the hierarchy of neural development. *Neuron* **1,** 3–13.

Jessell, T. M. (2000). Neuronal specification in the spinal cord: Inductive signals and transcriptional codes. *Nat. Rev. Genet.* **1,** 20–29.

Jia, C., and Halpern, M. (1996). Subclasses of vomeronasal receptor neurons: Differential expression of G proteins (Gi alpha 2 and G(o alpha)) and segregated projections to the accessory olfactory bulb. *Brain Res.* **719,** 117–128.

Jin, M., Guan, C. B., Jiang, Y. A., Chen, G., Zhao, C. T., Cui, K., Song, Y. Q., Wu, C. P., Poo, M. M., and Yuan, X. B. (2005). Ca2+-dependent regulation of rho GTPases triggers turning of nerve growth cones. *J. Neurosci.* **25,** 2338–2347.

Jin, Z., and Strittmatter, S. M. (1997). Rac1 mediates collapsin-1-induced growth cone collapse. *J. Neurosci.* **17,** 6256–6263.

Johnson, K. G., Ghose, A., Epstein, E., Lincecum, J., O'Connor, M. B., and Van Vactor, D. (2004). Axonal heparan sulfate proteoglycans regulate the distribution and efficiency of the repellent slit during midline axon guidance. *Curr. Biol* **14,** 499–504.

Joosten, E. A., Gribnau, A. A., and Dederen, P. J. (1989). Postnatal development of the corticospinal tract in the rat. An ultrastructural anterograde HRP study. *Anat. Embryol. (Berl.)* **179,** 449–456.

Kafitz, K. W., and Greer, C. A. (1997). Role of laminin in axonal extension from olfactory receptor cells. *J. Neurobiol.* **32,** 298–310.

Kafitz, K. W., and Greer, C. A. (1998). Differential expression of extracellular matrix and cell adhesion molecules in the olfactory nerve and glomerular layers of adult rats. *J. Neurobiol.* **34,** 271–282.

Kamiguchi, H., Long, K. E., Pendergast, M., Schaefer, A. W., Rapoport, I., Kirchhausen, T., and Lemmon, V. (1998). The neural cell adhesion molecule L1 interacts with the AP-2 adaptor and is endocytosed via the clathrin-mediated pathway. *J. Neurosci.* **18,** 5311–5321.

Kandel, E. R., Shwartz, J. H., and Jessell, T. M. (2000). "Principles of Neural Science." McGraw-Hill, New York.

Kantor, D. B., Chivatakarn, O., Peer, K. L., Oster, S. F., Inatani, M., Hansen, M. J., Flanagan, J. G., Yamaguchi, Y., Sretavan, D. W., Giger, R. J., and Kolodkin, A. L. (2004). Semaphorin 5A is a bifunctional axon guidance cue regulated by heparan and chondroitin sulfate proteoglycans. *Neuron* **44,** 961–975.

Keleman, K., Rajagopalan, S., Cleppien, D., Teis, D., Paiha, K., Huber, L. A., Technau, G. M., and Dickson, B. J. (2002). Comm sorts robo to control axon guidance at the Drosophila midline. *Cell* **110,** 415–427.

Keleman, K., Ribeiro, C., and Dickson, B. J. (2005). Comm function in commissural axon guidance: Cell-autonomous sorting of Robo *in vivo*. *Nat. Neurosci.* **8,** 156–163.

Kennedy, T. E., Serafini, T., de la Torre, J. R., and Tessier-Lavigne, M. (1994). Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. *Cell* **78,** 425–435.

Keshishian, H., and Bentley, D. (1983). Embryogenesis of peripheral nerve pathways in grasshopper legs. I. The initial nerve pathway to the CNS. *Dev. Biol.* **96,** 89–102.

Keverne, E. B. (1999). The vomeronasal organ. *Science* **286,** 716–720.

Key, B., and Akeson, R. A. (1990). Olfactory neurons express a unique glycosylated form of the neural cell adhesion molecule (N-CAM). *J. Cell Biol.* **110,** 1729–1743.

Kidd, T., Brose, K., Mitchell, K. J., Fetter, R. D., Tessier-Lavigne, M., Goodman, C. S., and Tear, G. (1998). Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. *Cell* **92,** 205–215.

Kidd, T., Bland, K. S., and Goodman, C. S. (1999). Slit is the midline repellent for the robo receptor in Drosophila. *Cell* **96,** 785–794.

Kinnunen, A., Kinnunen, T., Kaksonen, M., Nolo, R., Panula, P., and Rauvala, H. (1998). N-syndecan and HB-GAM (heparin-binding growth-associated molecule) associate with early axonal tracts in the rat brain. *Eur. J. Neurosci.* **10,** 635–648.

Klambt, C., Jacobs, J. R., and Goodman, C. S. (1991). The midline of the Drosophila central nervous system: A model for the genetic analysis of cell fate, cell migration, and growth cone guidance. *Cell* **64,** 801–815.

Knoll, B., Zarbalis, K., Wurst, W., and Drescher, U. (2001). A role for the EphA family in the topographic targeting of vomeronasal axons. *Development* **128,** 895–906.

Knoll, B., Schmidt, H., Andrews, W., Guthrie, S., Pini, A., Sundaresan, V., and Drescher, U. (2003). On the topographic targeting of basal vomeronasal axons through Slit-mediated chemorepulsion. *Development* **130,** 5073–5082.

Kobayashi, H., Koppel, A. M., Luo, Y., and Raper, J. A. (1997). A role for collapsin-1 in olfactory and cranial sensory axon guidance. *J. Neurosci.* **17,** 8339–8352.

Koch, M., Murrell, J. R., Hunter, D. D., Olson, P. F., Jin, W., Keene, D. R., Brunken, W. J., and Burgeson, R. E. (2000). A novel member of the netrin family, beta-netrin, shares homology with the beta chain of laminin: Identification, expression, and functional characterization. *J. Cell Biol.* **151,** 221–234.

Koester, S. E., and O'Leary, D. D. (1994). Development of projection neurons of the mammalian cerebral cortex. *Prog. Brain Res.* **102,** 207–215.

Kolodkin, A. L., Matthes, D. J., O'Connor, T. P., Patel, N. H., Admon, A., Bentley, D., and Goodman, C. S. (1992). Fasciclin IV: Sequence, expression, and function during growth cone guidance in the grasshopper embryo. *Neuron* **9,** 831–845.

Kolodkin, A. L., Matthes, D. J., and Goodman, C. S. (1993). The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules. *Cell* **75,** 1389–1399.

Kolodkin, A. L., Levengood, D. V., Rowe, E. G., Tai, Y. T., Giger, R. J., and Ginty, D. D. (1997). Neuropilin is a semaphorin III receptor. *Cell* **90,** 753–762.

Kornack, D. R., and Giger, R. J. (2005). Probing microtubule +TIPs: Regulation of axon branching. *Curr. Opin. Neurobiol.* **15,** 58–66.

Krause, M., Dent, E. W., Bear, J. E., Loureiro, J. J., and Gertler, F. B. (2003). Ena/VASP proteins: Regulators of the actin cytoskeleton and cell migration. *Annu. Rev. Cell Dev. Biol.* **19,** 541–564.

Krause, M., Leslie, J. D., Stewart, M., Lafuente, E. M., Valderrama, F., Jagannathan, R., Strasser, G. A., Rubinson, D. A., Liu, H., Way, M., Yaffe, M. B., Boussiotis, V. A., and Gertler, F. B. (2004). Lamellipodin, an Ena/VASP ligand, is implicated in the regulation of lamellipodial dynamics. *Dev. Cell* **7,** 571–583.

Krylova, O., Herreros, J., Cleverley, K. E., Ehler, E., Henriquez, J. P., Hughes, S. M., and Salinas, P. C. (2002). WNT-3, expressed by motoneurons, regulates terminal arborization of neurotrophin-3-responsive spinal sensory neurons. *Neuron* **35,** 1043–1056.

Kuhn, T. B., Brown, M. D., Wilcox, C. L., Raper, J. A., and Bamburg, J. R. (1999). Myelin and collapsin-1 induce motor neuron growth cone collapse through different pathways: Inhibition of collapse by opposing mutants of rac1. *J. Neurosci.* **19,** 1965–1975.

Kuiper, G. G., Enmark, E., Pelto-Huikko, M., Nilsson, S., and Gustafsson, J. A. (1996). Cloning of a novel receptor expressed in rat prostate and ovary. *Proc. Natl. Acad. Sci. USA* **93,** 5925–5930.

Kullander, K., and Klein, R. (2002). Mechanisms and functions of Eph and ephrin signalling. *Nat. Rev. Mol. Cell. Biol.* **3,** 475–486.

Kuwada, J. Y. (1986). Cell recognition by neuronal growth cones in a simple vertebrate embryo. *Science* **233,** 740–746.

Kwon, Y. T., Tsai, L. H., and Crandall, J. E. (1999). Callosal axon guidance defects in p35(-/-) mice. *J. Comp. Neurol.* **415,** 218–229.

Lafuente, E. M., van Puijenbroek, A. A., Krause, M., Carman, C. V., Freeman, G. J., Berezovskaya, A., Constantine, E., Springer, T. A., Gertler, F. B., and Boussiotis, V. A. (2004). RIAM, an Ena/VASP and Profilin ligand, interacts with Rap1-GTP and mediates Rap1-induced adhesion. *Dev. Cell* **7,** 585–595.

LaMantia, A. S., Colbert, M. C., and Linney, E. (1993). Retinoic acid induction and regional differentiation prefigure olfactory pathway formation in the mammalian forebrain. *Neuron* **10,** 1035–1048.

LaMantia, A. S., Bhasin, N., Rhodes, K., and Heemskerk, J. (2000). Mesenchymal/epithelial induction mediates olfactory pathway formation. *Neuron* **28,** 411–425.

Lander, A. D. (1998). Proteoglycans: Master regulators of molecular encounter? *Matrix Biol.* **17,** 465–472.

Lanier, L. M., Gates, M. A., Witke, W., Menzies, A. S., Wehman, A. M., Macklis, J. D., Kwiatkowski, D., Soriano, P., and Gertler, F. B. (1999). Mena is required for neurulation and commissure formation. *Neuron* **22,** 313–325.

Lebrand, C., Dent, E. W., Strasser, G. A., Lanier, L. M., Krause, M., Svitkina, T. M., Borisy, G. G., and Gertler, F. B. (2004). Critical role of Ena/VASP proteins for filopodia formation in neurons and in function downstream of netrin-1. *Neuron* **42,** 37–49.

Lee, K. J., Mendelsohn, M., and Jessell, T. M. (1998). Neuronal patterning by BMPs: A requirement for GDF7 in the generation of a discrete class of commissural interneurons in the mouse spinal cord. *Genes Dev.* **12,** 3394–3407.

Leegwater, P. A., van Driel, W., and van der Vliet, P. C. (1985). Recognition site of nuclear factor I, a sequence-specific DNA-binding protein from HeLa cells that stimulates adenovirus DNA replication. *EMBO J.* **4,** 1515–1521.

Letourneau, P. C., and Ressler, A. H. (1984). Inhibition of neurite initiation and growth by taxol. *J. Cell Biol.* **98,** 1355–1362.

Letourneau, P. C., Shattuck, T. A., and Ressler, A. H. (1986). Branching of sensory and sympathetic neurites *in vitro* is inhibited by treatment with taxol. *J. Neurosci.* **6,** 1912–1917.

Letourneau, P. C., Shattuck, T. A., and Ressler, A. H. (1987). "Pull" and "push" in neurite elongation: Observations on the effects of different concentrations of cytochalasin B and taxol *Cell Motil. Cytoskeleton* **8,** 193–209.

Leung, K. M., Taylor, J. S., and Chan, S. O. (2003). Enzymatic removal of chondroitin sulphates abolishes the age-related axon order in the optic tract of mouse embryos. *Eur. J. Neurosci.* **17,** 1755–1767.

Lewis, A. K., and Bridgman, P. C. (1992). Nerve growth cone lamellipodia contain two populations of actin filaments that differ in organization and polarity. *J. Cell Biol.* **119,** 1219–1243.

Li, H. S., Chen, J. H., Wu, W., Fagaly, T., Zhou, L., Yuan, W., Dupuis, S., Jiang, Z. H., Nash, W., Gick, C., Ornitz, D. M., Wu, J. Y., and Rao, Y. (1999). Vertebrate slit, a secreted ligand for the transmembrane protein roundabout, is a repellent for olfactory bulb axons. *Cell* **96,** 807–818.

Lim, Y. S., and Wadsworth, W. G. (2002). Identification of domains of netrin UNC-6 that mediate attractive and repulsive guidance and responses from cells and growth cones. *J. Neurosci.* **22,** 7080–7087.

Lin, C. H., and Forscher, P. (1993). Cytoskeletal remodeling during growth cone-target interactions. *J. Cell Biol.* **121,** 1369–1383.

Lin, C. H., and Forscher, P. (1995). Growth cone advance is inversely proportional to retrograde F-actin flow. *Neuron* **14,** 763–771.

Lin, C. H., Thompson, C. A., and Forscher, P. (1994). Cytoskeletal reorganization underlying growth cone motility. *Curr. Opin. Neurobiol.* **4,** 640–647.

Little, M., Rumballe, B., Georgas, K., Yamada, T., and Teasdale, R. D. (2002). Conserved modularity and potential for alternate splicing in mouse and human Slit genes. *Int J. Dev. Biol.* **46,** 385–391.

Liu, A., Majumdar, A., Schauerte, H. E., Haffter, P., and Drummond, I. A. (2000). Zebrafish wnt4b expression in the floor plate is altered in sonic hedgehog and gli-2 mutants. *Mech. Dev.* **91,** 409–413.

Liu, B. P., and Strittmatter, S. M. (2001). Semaphorin-mediated axonal guidance via Rho-related G proteins. *Curr. Opin. Cell Biol.* **13,** 619–626.

Liu, S. F., Jiang, Y. L., and Du, L. X. (2003). [Studies of BMPR-IB and BMP15 as candidate genes for fecundity in little tailed han sheep]. *Yi Chuan Xue Bao* **30,** 755–760.

Livesey, F. J., and Hunt, S. P. (1997). Netrin and netrin receptor expression in the embryonic mammalian nervous system suggests roles in retinal, striatal, nigral, and cerebellar development. *Mol. Cell Neurosci.* **8,** 417–429.

Long, H., Sabatier, C., Ma, L., Plump, A., Yuan, W., Ornitz, D. M., Tamada, A., Murakami, F., Goodman, C. S., and Tessier-Lavigne, M. (2004). Conserved roles for Slit and Robo proteins in midline commissural axon guidance. *Neuron* **42,** 213–223.

Luduena, M. A., and Wessells, N. K. (1973). Cell locomotion, nerve elongation, and microfilaments. *Dev. Biol.* **30,** 427–440.

Lumsden, A. G., and Davies, A. M. (1983). Earliest sensory nerve fibres are guided to peripheral targets by attractants other than nerve growth factor. *Nature* **306,** 786–788.

Lundquist, E. A. (2003). Rac proteins and the control of axon development. *Curr. Opin. Neurobiol.* **13,** 384–390.

Luo, L., Jan, L. Y., and Jan, Y. N. (1997). Rho family GTP-binding proteins in growth cone signalling. *Curr. Opin. Neurobiol.* **7,** 81–86.

Luo, Y., Raible, D., and Raper, J. A. (1993). Collapsin: A protein in brain that induces the collapse and paralysis of neuronal growth cones. *Cell* **75,** 217–227.

Lustig, M., Erskine, L., Mason, C. A., Grumet, M., and Sakurai, T. (2001). Nr-CAM expression in the developing mouse nervous system: Ventral midline structures, specific fiber tracts, and neuropilar regions. *J. Comp. Neurol.* **434,** 13–28.

Lyuksyutova, A. I., Lu, C. C., Milanesio, N., King, L. A., Guo, N., Wang, Y., Nathans, J., Tessier-Lavigne, M., and Zou, Y. (2003). Anterior-posterior guidance of commissural axons by Wnt-frizzled signaling. *Science* **302,** 1984–1988.

Maisonpierre, P. C., Barrezueta, N. X., and Yancopoulos, G. D. (1993). Ehk-1 and Ehk-2: Two novel members of the Eph receptor-like tyrosine kinase family with distinctive structures and neuronal expression. *Oncogene* **8,** 3277–3288.

Mallavarapu, A., and Mitchison, T. (1999). Regulated actin cytoskeleton assembly at filopodium tips controls their extension and retraction. *J. Cell Biol.* **146,** 1097–1106.

Mann, F., Peuckert, C., Dehner, F., Zhou, R., and Bolz, J. (2002a). Ephrins regulate the formation of terminal axonal arbors during the development of thalamocortical projections. *Development* **129,** 3945–3955.

Mann, F., Ray, S., Harris, W., and Holt, C. (2002b). Topographic mapping in dorsoventral axis of the Xenopus retinotectal system depends on signaling through ephrin-B ligands. *Neuron* **35,** 461–473.

Marcus, R. C., and Mason, C. A. (1995). The first retinal axon growth in the mouse optic chiasm: Axon patterning and the cellular environment. *J. Neurosci.* **15,** 6389–6402.

Marcus, R. C., Blazeski, R., Godement, P., and Mason, C. A. (1995). Retinal axon divergence in the optic chiasm: Uncrossed axons diverge from crossed axons within a midline glial specialization. *J. Neurosci.* **15,** 3716–3729.

Margolis, R. K., Rauch, U., Maurel, P., and Margolis, R. U. (1996). Neurocan and phosphacan: Two major nervous tissue-specific chondroitin sulfate proteoglycans. *Perspect. Dev. Neurobiol.* **3,** 273–290.

Marillat, V., Cases, O., Nguyen-Ba-Charvet, K. T., Tessier-Lavigne, M., Sotelo, C., and Chedotal, A. (2002). Spatiotemporal expression patterns of slit and robo genes in the rat brain. *J. Comp. Neurol.* **442,** 130–155.

Marsh, L., and Letourneau, P. C. (1984). Growth of neurites without filopodial or lamellipodial activity in the presence of cytochalasin B. *J. Cell Biol.* **99,** 2041–2047.

Marti, E., and Bovolenta, P. (2002). Sonic hedgehog in CNS development: One signal, multiple outputs. *Trends Neurosci.* **25,** 89–96.

Mason, C. A., and Sretavan, D. W. (1997). Glia, neurons, and axon pathfinding during optic chiasm development. *Curr. Opin. Neurobiol.* **7,** 647–653.

Mason, C. A., and Wang, L. C. (1997). Growth cone form is behavior-specific and, consequently, position-specific along the retinal axon pathway. *J. Neurosci.* **17,** 1086–1100.

Mastick, G. S., and Easter, S. S., Jr. (1996). Initial organization of neurons and tracts in the embryonic mouse fore- and midbrain. *Dev. Biol.* **173,** 79–94.

Matheson, S. F., and Levine, R. B. (1999). Steroid hormone enhancement of neurite outgrowth in identified insect motor neurons involves specific effects on growth cone form and function. *J. Neurobiol.* **38,** 27–45.

Matise, M. P., Lustig, M., Sakurai, T., Grumet, M., and Joyner, A. L. (1999). Ventral midline cells are required for the local control of commissural axon guidance in the mouse spinal cord. *Development* **126,** 3649–3659.

Matsunami, H., and Buck, L. B. (1997). A multigene family encoding a diverse array of putative pheromone receptors in mammals. *Cell* **90,** 775–784.

McConnell, S. K., Ghosh, A., and Shatz, C. J. (1989). Subplate neurons pioneer the first axon pathway from the cerebral cortex. *Science* **245,** 978–982.

McConnell, S. K., Ghosh, A., and Shatz, C. J. (1994). Subplate pioneers and the formation of descending connections from cerebral cortex. *J. Neurosci.* **14,** 1892–1907.

McLaughlin, T., Hindges, R., and O'Leary, D. D. (2003a). Regulation of axial patterning of the retina and its topographic mapping in the brain. *Curr. Opin. Neurobiol.* **13,** 57–69.

McLaughlin, T., Hindges, R., Yates, P. A., and O'Leary, D. D. (2003b). Bifunctional action of ephrin-B1 as a repellent and attractant to control bidirectional branch extension in dorsal-ventral retinotopic mapping. *Development* **130,** 2407–2418.

Mehlen, P., and Mazelin, L. (2003). The dependence receptors DCC and UNC5H as a link between neuronal guidance and survival. *Biol. Cell* **95,** 425–436.

Meixner, A., Haverkamp, S., Wassle, H., Fuhrer, S., Thalhammer, J., Kropf, N., Bittner, R. E., Lassmann, H., Wiche, G., and Propst, F. (2000). MAP1B is required for axon guidance and Is involved in the development of the central and peripheral nervous system. *J. Cell Biol.* **151,** 1169–1178.

Mellitzer, G., Xu, Q., and Wilkinson, D. G. (1999). Eph receptors and ephrins restrict cell intermingling and communication. *Nature* **400,** 77–81.

Meriane, M., Tcherkezian, J., Webber, C. A., Danek, E. I., Triki, I., McFarlane, S., Bloch-Gallego, E., and Lamarche-Vane, N. (2004). Phosphorylation of DCC by Fyn mediates Netrin-1 signaling in growth cone guidance. *J. Cell Biol.* **167,** 687–698.

Metin, C., Deleglise, D., Serafini, T., Kennedy, T. E., and Tessier-Lavigne, M. (1997). A role for netrin-1 in the guidance of cortical efferents. *Development* **124,** 5063–5074.

Mey, J., and Thanos, S. (1992). Development of the visual system of the chick—a review. *J. Hirnforsch.* **33,** 673–702.

Ming, G. L., Wong, S. T., Henley, J., Yuan, X. B., Song, H. J., Spitzer, N. C., and Poo, M. M. (2002). Adaptation in the chemotactic guidance of nerve growth cones. *Nature* **417,** 411–418.

Miragall, F., and Dermietzel, R. (1992). Immunocytochemical localization of cell adhesion molecules in the developing and mature olfactory system. *Microsc. Res. Tech.* **23,** 157–172.

Miragall, F., Kadmon, G., and Schachner, M. (1989). Expression of L1 and N-CAM cell adhesion molecules during development of the mouse olfactory system. *Dev. Biol.* **135,** 272–286.

Miranda, R., Sohrabji, F., Singh, M., and Toran-Allerand, D. (1996). Nerve growth factor (NGF) regulation of estrogen receptors in explant cultures of the developing forebrain. *J. Neurobiol.* **31,** 77–87.

Miranda, R. C., Sohrabji, F., and Toran-Allerand, C. D. (1993). Neuronal colocalization of mRNAs for neurotrophins and their receptors in the developing central nervous system suggests a potential for autocrine interactions. *Proc. Natl. Acad. Sci. USA* **90,** 6439–6443.

Mitchison, T., and Kirschner, M. (1988). Cytoskeletal dynamics and nerve growth. *Neuron* **1,** 761–772.

Mombaerts, P. (1996). Targeting olfaction. *Curr. Opin. Neurobiol.* **6,** 481–486.

Mombaerts, P., Wang, F., Dulac, C., Chao, S. K., Nemes, A., Mendelsohn, M., Edmondson, J., and Axel, R. (1996). Visualizing an olfactory sensory map. *Cell* **87,** 675–686.

Montag-Sallaz, M., Schachner, M., and Montag, D. (2002). Misguided axonal projections, neural cell adhesion molecule 180 mRNA upregulation, and altered behavior in mice deficient for the close homolog of L1. *Mol. Cell. Biol* **22,** 7967–7981.

Morgenstern, D. A., Asher, R. A., and Fawcett, J. W. (2002). Chondroitin sulphate proteoglycans in the CNS injury response. *Prog. Brain Res.* **137,** 313–332.

Mori, K., Ikeda, J., and Hayaishi, O. (1990). Monoclonal antibody R2D5 reveals midsagittal radial glial system in postnatally developing and adult brainstem. *Proc. Natl. Acad. Sci. USA* **87,** 5489–5493.

Mueller, B. K. (1999). Growth cone guidance: First steps towards a deeper understanding. *Annu. Rev. Neurosci.* **22,** 351–388.

Mui, S. H., Hindges, R., O'Leary, D. D., Lemke, G., and Bertuzzi, S. (2002). The homeodomain protein Vax2 patterns the dorsoventral and nasotemporal axes of the eye. *Development* **129,** 797–804.

Mullins, R. D., Heuser, J. A., and Pollard, T. D. (1998). The interaction of Arp2/3 complex with actin: Nucleation, high affinity pointed end capping, and formation of branching networks of filaments. *Proc. Natl. Acad. Sci. USA* **95,** 6181–6186.

Myat, A., Henry, P., McCabe, V., Flintoft, L., Rotin, D., and Tear, G. (2002). Drosophila Nedd4, a ubiquitin ligase, is recruited by Commissureless to control cell surface levels of the roundabout receptor. *Neuron* **35,** 447–459.

Nagata, K., Guggenheimer, R. A., Enomoto, T., Lichy, J. H., and Hurwitz, J. (1982). Adenovirus DNA replication *in vitro*: Identification of a host factor that stimulates synthesis of the preterminal protein-dCMP complex. *Proc. Natl. Acad. Sci. USA* **79,** 6438–6442.

Nagata, K., Guggenheimer, R. A., and Hurwitz, J. (1983). Specific binding of a cellular DNA replication protein to the origin of replication of adenovirus DNA. *Proc. Natl. Acad. Sci. USA* **80,** 6177–6181.

Nakagawa, H., Miki, H., Ito, M., Ohashi, K., Takenawa, T., and Miyamoto, S. (2001). N-WASP, WAVE and Mena play different roles in the organization of actin cytoskeleton in lamellipodia. *J. Cell Sci.* **114,** 1555–1565.

Nakamura, F., Kalb, R. G., and Strittmatter, S. M. (2000). Molecular basis of semaphorin-mediated axon guidance. *J. Neurobiol.* **44,** 219–229.

Nakashiba, T., Nishimura, S., Ikeda, T., and Itohara, S. (2002). Complementary expression and neurite outgrowth activity of netrin-G subfamily members. *Mech. Dev.* **111,** 47–60.

Narasimhan, K. (2004). Assaying axon sensitivity. *Nat. Neurosci.* **7,** 574.

Neely, M. D., and Gesemann, M. (1994). Disruption of microfilaments in growth cones following depolarization and calcium influx. *J. Neurosci.* **14,** 7511–7520.

Nguyen-Ba-Charvet, K. T., Brose, K., Marillat, V., Sotelo, C., Tessier-Lavigne, M., and Chedotal, A. (2001). Sensory axon response to substrate-bound Slit2 is modulated by laminin and cyclic GMP. *Mol. Cell Neurosci.* **17,** 1048–1058.

Nguyen-Ba-Charvet, K. T., Plump, A. S., Tessier-Lavigne, M., and Chedotal, A. (2002). Slit1 and slit2 proteins control the development of the lateral olfactory tract. *J. Neurosci.* **22,** 5473–5480.

Niclou, S. P., Jia, L., and Raper, J. A. (2000). Slit2 is a repellent for retinal ganglion cell axons. *J. Neurosci.* **20,** 4962–4974.

Nordlander, R. H., Singer, J. F., Beck, R., and Singer, M. (1981). An ultrastructural examination of early ventral root formation in amphibia. *J. Comp. Neurol.* **199,** 535–551.

Nowock, J., Borgmeyer, U., Puschel, A. W., Rupp, R. A., and Sippel, A. E. (1985). The TGGCA protein binds to the MMTV-LTR, the adenovirus origin of replication, and the BK virus enhancer. *Nucleic Acids Res.* **13,** 2045–2061.

Nural, H. F., and Mastick, G. S. (2004). Pax6 guides a relay of pioneer longitudinal axons in the embryonic mouse forebrain. *J. Comp. Neurol.* **479,** 399–409.

Oakley, R. A., and Tosney, K. W. (1991). Peanut agglutinin and chondroitin-6-sulfate are molecular markers for tissues that act as barriers to axon advance in the avian embryo. *Dev. Biol.* **147,** 187–206.

O'Connor, T. P., and Bentley, D. (1993). Accumulation of actin in subsets of pioneer growth cone filopodia in response to neural and epithelial guidance cues in situ. *J. Cell Biol.* **123,** 935–948.

O'Leary, D. D., Bicknese, A. R., De Carlos, J. A., Heffner, C. D., Koester, S. E., Kutka, L. J., and Terashima, T. (1990). Target selection by cortical axons: Alternative mechanisms to establish axonal connections in the developing brain. *Cold Spring Harb. Symp. Quant. Biol.* **55,** 453–468.

O'Leary, D. M., Gerfen, C. R., and Cowan, W. M. (1983). The development and restriction of the ipsilateral retinofugal projection in the chick. *Brain Res.* **312,** 93–109.

Orioli, D., Henkemeyer, M., Lemke, G., Klein, R., and Pawson, T. (1996). Sek4 and Nuk receptors cooperate in guidance of commissural axons and in palate formation. *EMBO J.* **15,** 6035–6049.

Ozaki, H. S., and Wahlsten, D. (1998). Timing and origin of the first cortical axons to project through the corpus callosum and the subsequent emergence of callosal projection cells in mouse. *J. Comp. Neurol.* **400,** 197–206.

Ozdinler, P. H., and Erzurumlu, R. S. (2002). Slit2, a branching-arborization factor for sensory axons in the Mammalian CNS. *J. Neurosci.* **22,** 4540–4549.

Packard, M., Koo, E. S., Gorczyca, M., Sharpe, J., Cumberledge, S., and Budnik, V. (2002). The Drosophila Wnt, wingless, provides an essential signal for pre- and postsynaptic differentiation. *Cell* **111,** 319–330.

Palmer, A., and Klein, R. (2003). Multiple roles of ephrins in morphogenesis, neuronal networking, and brain function. *Genes Dev.* **17,** 1429–5140.

Pasterkamp, R. J., and Kolodkin, A. L. (2003). Semaphorin junction: Making tracks toward neural connectivity. *Curr. Opin. Neurobiol.* **13,** 79–89.

Pasterkamp, R. J., De Winter, F., Holtmaat, A. J., and Verhaagen, J. (1998). Evidence for a role of the chemorepellent semaphorin III and its receptor neuropilin-1 in the regeneration of primary olfactory axons. *J. Neurosci.* **18,** 9962–9976.

Pasterkamp, R. J., Ruitenberg, M. J., and Verhaagen, J. (1999). Semaphorins and their receptors in olfactory axon guidance. *Cell Mol. Biol. (Noisy-le-grand)* **45,** 763–779.

Pasterkamp, R. J., Peschon, J. J., Spriggs, M. K., and Kolodkin, A. L. (2003). Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. *Nature* **424,** 398–405.

Paves, H., and Saarma, M. (1997). Neurotrophins as *in vitro* growth cone guidance molecules for embryonic sensory neurons. *Cell Tissue Res.* **290,** 285–297.

Piper, M., Georgas, K., Yamada, T., and Little, M. (2000). Expression of the vertebrate Slit gene family and their putative receptors, the Robo genes, in the developing murine kidney. *Mech. Dev.* **94,** 213–217.

Pires-Neto, M. A., Braga-De-Souza, S., and Lent, R. (1998). Molecular tunnels and boundaries for growing axons in the anterior commissure of hamster embryos. *J. Comp. Neurol.* **399,** 176–188.

Pistor, S., Chakraborty, T., Walter, U., and Wehland, J. (1995). The bacterial actin nucleator protein ActA of Listeria monocytogenes contains multiple binding sites for host microfilament proteins. *Curr. Biol* **5,** 517–525.

Pittman, A., and Chien, C. B. (2002). Understanding dorsoventral topography: Backwards and forwards. *Neuron* **35,** 409–411.

Placzek, M., Tessier-Lavigne, M., Jessell, T., and Dodd, J. (1990). Orientation of commissural axons *in vitro* in response to a floor plate-derived chemoattractant. *Development* **110,** 19–30.

Plump, A. S., Erskine, L., Sabatier, C., Brose, K., Epstein, C. J., Goodman, C. S., Mason, C. A., and Tessier-Lavigne, M. (2002). Slit1 and Slit2 cooperate to prevent premature midline crossing of retinal axons in the mouse visual system. *Neuron* **33,** 219–232.

Puche, A. C., and Key, B. (1996). N-acetyl-lactosamine in the rat olfactory system: Expression and potential role in neurite growth. *J. Comp. Neurol.* **364,** 267–278.

Puche, A. C., Poirier, F., Hair, M., Bartlett, P. F., and Key, B. (1996). Role of galectin-1 in the developing mouse olfactory system. *Dev. Biol.* **179,** 274–287.

Raabe, E. H., Yoshida, K., and Schwarting, G. A. (1997). Differential laminin isoform expression in the developing rat olfactory system. *Brain Res. Dev. Brain Res.* **101,** 187–196.

Raper, J. A. (2000). Semaphorins and their receptors in vertebrates and invertebrates. *Curr. Opin. Neurobiol.* **10,** 88–94.

Raper, J. A., Bastiani, M., and Goodman, C. S. (1983a). Pathfinding by neuronal growth cones in grasshopper embryos. I. Divergent choices made by the growth cones of sibling neurons. *J. Neurosci.* **3,** 20–30.

Raper, J. A., Bastiani, M., and Goodman, C. S. (1983b). Pathfinding by neuronal growth cones in grasshopper embryos. II. Selective fasciculation onto specific axonal pathways. *J. Neurosci.* **3,** 31–41.

Raper, J. A., Bastiani, M. J., and Goodman, C. S. (1984). Pathfinding by neuronal growth cones in grasshopper embryos. IV. The effects of ablating the A and P axons upon the behavior of the G growth cone. *J. Neurosci.* **4,** 2329–2345.

Rasband, K., Hardy, M., and Chien, C. B. (2003). Generating X: Formation of the optic chiasm. *Neuron* **39,** 885–888.

Rash, B. G., and Richards, L. J. (2001). A role for cingulate pioneering axons in the development of the corpus callosum. *J. Comp. Neurol.* **434,** 147–157.

Renzi, M. J., Wexler, T. L., and Raper, J. A. (2000). Olfactory sensory axons expressing a dominant-negative semaphorin receptor enter the CNS early and overshoot their target. *Neuron* **28,** 437–447.

Ressler, K. J., Sullivan, S. L., and Buck, L. B. (1994). A molecular dissection of spatial patterning in the olfactory system. *Curr. Opin. Neurobiol.* **4,** 588–596.

Rice, D. S., Williams, R. W., and Goldowitz, D. (1995). Genetic control of retinal projections in inbred strains of albino mice. *J. Comp. Neurol.* **354,** 459–469.

Richards, L. J., Koester, S. E., Tuttle, R., and O'Leary, D. D. (1997). Directed growth of early cortical axons is influenced by a chemoattractant released from an intermediate target. *J. Neurosci.* **17,** 2445–2458.

Richards, L. J., Plachez, C., and Ren, T. (2004). Mechanisms regulating the development of the corpus callosum and its agenesis in mouse and human. *Clin. Genet.* **66,** 276–289.

Ringstedt, T., Braisted, J. E., Brose, K., Kidd, T., Goodman, C., Tessier-Lavigne, M., and O'Leary, D. D. (2000). Slit inhibition of retinal axon growth and its role in retinal axon pathfinding and innervation patterns in the diencephalon. *J. Neurosci.* **20,** 4983–4991.

Rivas, R. J., and Hatten, M. E. (1995). Motility and cytoskeletal organization of migrating cerebellar granule neurons. *J. Neurosci.* **15,** 981–989.

Rochlin, M. W., Wickline, K. M., and Bridgman, P. C. (1996). Microtubule stability decreases axon elongation but not axoplasm production. *J. Neurosci.* **16,** 3236–3246.

Rochlin, M. W., Dailey, M. E., and Bridgman, P. C. (1999). Polymerizing microtubules activate site-directed F-actin assembly in nerve growth cones. *Mol. Biol. Cell* **10,** 2309–2327.

Rodriguez, O. C., Schaefer, A. W., Mandato, C. A., Forscher, P., Bement, W. M., and Waterman-Storer, C. M. (2003). Conserved microtubule-actin interactions in cell movement and morphogenesis. *Nat. Cell Biol.* **5,** 599–609.

Roskies, A. L., and O'Leary, D. D. (1994). Control of topographic retinal axon branching by inhibitory membrane-bound molecules. *Science* **265,** 799–803.

Rosoff, W. J., Urbach, J. S., Esrick, M. A., McAllister, R. G., Richards, L. J., and Goodhill, G. J. (2004). A new chemotaxis assay shows the extreme sensitivity of axons to molecular gradients. *Nat. Neurosci.* **7,** 678–682.

Ross, L. S., Parrett, T., and Easter, S. S., Jr. (1992). Axonogenesis and morphogenesis in the embryonic zebrafish brain. *J. Neurosci.* **12,** 467–482.

Rothberg, J. M., Jacobs, J. R., Goodman, C. S., and Artavanis-Tsakonas, S. (1990). slit: An extracellular protein necessary for development of midline glia and commissural axon pathways contains both EGF and LRR domains. *Genes Dev.* **4,** 2169–2187.

Ruthazer, E. S., and Cline, H. T. (2004). Insights into activity-dependent map formation from the retinotectal system: A middle-of-the-brain perspective. *J. Neurobiol.* **59,** 134–146.

Ryba, N. J., and Tirindelli, R. (1997). A new multigene family of putative pheromone receptors. *Neuron* **19,** 371–379.

Sabatier, C., Plump, A. S., Le, M., Brose, K., Tamada, A., Murakami, F., Lee, E. Y., and Tessier-Lavigne, M. (2004). The divergent Robo family protein rig-1/Robo3 is a negative regulator of slit responsiveness required for midline crossing by commissural axons. *Cell* **117,** 157–169.

Sabry, J. H., O'Connor, T. P., Evans, L., Toroian-Raymond, A., Kirschner, M., and Bentley, D. (1991). Microtubule behavior during guidance of pioneer neuron growth cones in situ. *J. Cell Biol.* **115,** 381–395.

Salinas, P. C. (2003). The morphogen sonic hedgehog collaborates with netrin-1 to guide axons in the spinal cord. *Trends Neurosci.* **26,** 641–643.

Sanchez, D., Ganfornina, M. D., and Bastiani, M. J. (1995). Developmental expression of the lipocalin Lazarillo and its role in axonal pathfinding in the grasshopper embryo. *Development* **121,** 135–147.

Sanchez, D., Ganfornina, M. D., and Bastiani, M. J. (2000). Lazarillo, a neuronal lipocalin in grasshoppers with a role in axon guidance. *Biochim. Biophys. Acta* **1482,** 102–109.

Sasaki, Y., Cheng, C., Uchida, Y., Nakajima, O., Ohshima, T., Yagi, T., Taniguchi, M., Nakayama, T., Kishida, R., Kudo, Y., Ohno, S., Nakamura, F., and Goshima, Y. (2002). Fyn and Cdk5 mediate semaphorin-3A signaling, which is involved in regulation of dendrite orientation in cerebral cortex. *Neuron* **35,** 907–920.

Sato, Y., Hirata, T., Ogawa, M., and Fujisawa, H. (1998). Requirement for early-generated neurons recognized by monoclonal antibody lot1 in the formation of lateral olfactory tract. *J. Neurosci.* **18,** 7800–7810.

Schnapp, B. J., Vale, R. D., Sheetz, M. P., and Reese, T. S. (1986). Microtubules and the mechanism of directed organelle movement. *Ann. N Y Acad. Sci.* **466,** 909–918.

Schwarting, G. A., Kostek, C., Ahmad, N., Dibble, C., Pays, L., and Puschel, A. W. (2000). Semaphorin 3A is required for guidance of olfactory axons in mice. *J. Neurosci.* **20,** 7691–7697.

Schwob, J. E., and Price, J. L. (1984). The development of lamination of afferent fibers to the olfactory cortex in rats, with additional observations in the adult. *J. Comp. Neurol.* **223,** 203–222.

Seeger, M., Tear, G., Ferres-Marco, D., and Goodman, C. S. (1993). Mutations affecting growth cone guidance in Drosophila: Genes necessary for guidance toward or away from the midline. *Neuron* **10,** 409–426.

Semaphorin Nomenclature Committee (1999). Unified nomenclature for the semaphorins/collapsins. *Cell* **97,** 551–552.

Serafini, T., Kennedy, T. E., Galko, M. J., Mirzayan, C., Jessell, T. M., and Tessier-Lavigne, M. (1994). The netrins define a family of axon outgrowth-promoting proteins homologous to C. elegans UNC-6. *Cell* **78,** 409–424.

Serafini, T., Colamarino, S. A., Leonardo, E. D., Wang, H., Beddington, R., Skarnes, W. C., and Tessier-Lavigne, M. (1996). Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. *Cell* **87,** 1001–1014.

Shen, K., and Bargmann, C. I. (2003). The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in C. elegans. *Cell* **112,** 619–630.

Shen, K., Fetter, R. D., and Bargmann, C. I. (2004). Synaptic specificity is generated by the synaptic guidepost protein SYG-2 and its receptor, SYG-1. *Cell* **116,** 869–881.

Shen, Y., Mani, S., Donovan, S. L., Schwob, J. E., and Meiri, K. F. (2002). Growth-associated protein-43 is required for commissural axon guidance in the developing vertebrate nervous system. *J. Neurosci.* **22,** 239–247.

Shewan, D., Dwivedy, A., Anderson, R., and Holt, C. E. (2002). Age-related changes underlie switch in netrin-1 responsiveness as growth cones advance along visual pathway. *Nat. Neurosci.* **5,** 955–962.

Shipley, M. T., and Ennis, M. (1996). Functional organization of olfactory system. *J. Neurobiol.* **30,** 123–176.

Shipley, M. Y., McLean, J. H., and Ennis, M. (1995). Olfactory system. *In* "The Rat Nervous System" (G. Paxinos, Ed.), 2nd edn. Academic Press, San Diego.

Shu, T., and Richards, L. J. (2001). Cortical axon guidance by the glial wedge during the development of the corpus callosum. *J. Neurosci.* **21,** 2749–2758.

Shu, T., Valentino, K. M., Seaman, C., Cooper, H. M., and Richards, L. J. (2000). Expression of the netrin-1 receptor, deleted in colorectal cancer (DCC), is largely confined to projecting neurons in the developing forebrain. *J. Comp. Neurol.* **416,** 201–212.

Shu, T., Butz, K. G., Plachez, C., Gronostajski, R. M., and Richards, L. J. (2003a). Abnormal development of forebrain midline glia and commissural projections in Nfia knock-out mice. *J. Neurosci.* **23,** 203–212.

Shu, T., Li, Y., Keller, A., and Richards, L. J. (2003b). The glial sling is a migratory population of developing neurons. *Development* **130,** 2929–2937.

Shu, T., Puche, A. C., and Richards, L. J. (2003c). Development of midline glial populations at the corticoseptal boundary. *J. Neurobiol.* **57,** 81–94.

Shu, T., Sundaresan, V., McCarthy, M. M., and Richards, L. J. (2003d). Slit2 guides both precrossing and postcrossing callosal axons at the midline *in vivo*. *J. Neurosci.* **23,** 8176–8184.

Silver, J. (1994). Inhibitory molecules in development and regeneration. *J. Neurol.* **242,** S22–24.

Silver, J., and Ogawa, M. Y. (1983). Postnatally induced formation of the corpus callosum in acallosal mice on glia-coated cellulose bridges. *Science* **220,** 1067–1069.

Silver, J., and Sapiro, J. (1981). Axonal guidance during development of the optic nerve: The role of pigmented epithelia and other extrinsic factors. *J. Comp. Neurol.* **202,** 521–538.

Silver, J., and Sidman, R. L. (1980). A mechanism for the guidance and topographic patterning of retinal ganglion cell axons. *J. Comp. Neurol.* **189,** 101–111.

Silver, J., Lorenz, S. E., Wahlsten, D., and Coughlin, J. (1982). Axonal guidance during development of the great cerebral commissures: Descriptive and experimental studies, *in vivo*, on the role of preformed glial pathways. *J. Comp. Neurol.* **210,** 10–29.

Silver, J., Edwards, M. A., and Levitt, P. (1993). Immunocytochemical demonstration of early appearing astroglial structures that form boundaries and pathways along axon tracts in the fetal brain. *J. Comp. Neurol.* **328,** 415–436.

Simerly, R. B. (2002). Wired for reproduction: Organization and development of sexually dimorphic circuits in the mammalian forebrain. *Annu. Rev. Neurosci.* **25,** 507–536.

Singer, M., Nordlander, R. H., and Egar, M. (1979). Axonal guidance during embryogenesis and regeneration in the spinal cord of the newt: The blueprint hypothesis of neuronal pathway patterning. *J. Comp. Neurol.* **185,** 1–21.

Singh, M., Setalo, G., Jr., Guan, X., Warren, M., and Toran-Allerand, C. D. (1999). Estrogen-induced activation of mitogen-activated protein kinase in cerebral cortical explants: Convergence of estrogen and neurotrophin signaling pathways. *J. Neurosci.* **19,** 1179–1188.

Skynner, M. J., Sim, J. A., and Herbison, A. E. (1999). Detection of estrogen receptor alpha and beta messenger ribonucleic acids in adult gonadotropin-releasing hormone neurons. *Endocrinology* **140,** 5195–5201.

Smith, G. A., Theriot, J. A., and Portnoy, D. A. (1996). The tandem repeat domain in the Listeria monocytogenes ActA protein controls the rate of actin-based motility, the percentage of moving bacteria, and the localization of vasodilator-stimulated phosphoprotein and profilin. *J. Cell Biol.* **135,** 647–660.

Soker, S., Takashima, S., Miao, H. Q., Neufeld, G., and Klagsbrun, M. (1998). Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. *Cell* **92,** 735–745.

Song, H., Ming, G., He, Z., Lehmann, M., McKerracher, L., Tessier-Lavigne, M., and Poo, M. (1998). Conversion of neuronal growth cone responses from repulsion to attraction by cyclic nucleotides. *Science* **281,** 1515–1518.

Song, H. J., Ming, G. L., and Poo, M. M. (1997). cAMP-induced switching in turning direction of nerve growth cones. *Nature* **388,** 275–279.

Soriano, E., Del Rio, J. A., Martinez, A., and Super, H. (1994). Organization of the embryonic and early postnatal murine hippocampus. I. Immunocytochemical characterization of neuronal populations in the subplate and marginal zone. *J. Comp. Neurol.* **342,** 571–595.

Spassky, N., de Castro, F., Le Bras, B., Heydon, K., Queraud-Le Saux, F., Bloch-Gallego, E., Chedotal, A., Zalc, B., and Thomas, J. L. (2002). Directional guidance of oligodendroglial migration by class 3 semaphorins and netrin-1. *J. Neurosci.* **22,** 5992–6004.

Sperry, R. (1982). Some effects of disconnecting the cerebral hemispheres. *Science* **217,** 1223–1226.

Sperry, R. W. (1963). Chemoaffinity In The Orderly Growth Of Nerve Fiber Patterns And Connections. *Proc. Natl. Acad. Sci. USA* **50,** 703–710.

Sperry, R. W. (1968). Hemisphere deconnection and unity in conscious awareness. *Am. Psychol.* **23,** 723–733.

Sretavan, D. W. (1993). Pathfinding at the mammalian optic chiasm. *Curr. Opin. Neurobiol.* **3,** 45–52.

Sretavan, D. W., and Reichardt, L. F. (1993). Time-lapse video analysis of retinal ganglion cell axon pathfinding at the mammalian optic chiasm: Growth cone guidance using intrinsic chiasm cues. *Neuron* **10,** 761–777.

Sretavan, D. W., Feng, L., Pure, E., and Reichardt, L. F. (1994). Embryonic neurons of the developing optic chiasm express L1 and CD44, cell surface molecules with opposing effects on retinal axon growth. *Neuron* **12,** 957–975.

Sretavan, D. W., Pure, E., Siegel, M. W., and Reichardt, L. F. (1995). Disruption of retinal axon ingrowth by ablation of embryonic mouse optic chiasm neurons. *Science* **269,** 98–101.

St John, J. A., and Key, B. (2001). EphB2 and two of its ligands have dynamic protein expression patterns in the developing olfactory system. *Brain Res. Dev. Brain Res.* **126,** 43–56.

St John, J. A., Tisay, K. T., Caras, I. W., and Key, B. (2000). Expression of EphA5 during development of the olfactory nerve pathway in rat. *J. Comp. Neurol.* **416,** 540–550.

St John, J. A., Pasquale, E. B., and Key, B. (2002). EphA receptors and ephrin-A ligands exhibit highly regulated spatial and temporal expression patterns in the developing olfactory system. *Brain Res. Dev. Brain Res.* **138,** 1–14.

Steele-Perkins, G., Plachez, C., Butz, K. G., Yang, G., Bachurski, C. J., Kinsman, S. L., Litwack, E. D., Richards, L. J., and Gronostajski, R. M. (2005). The transcription factor gene Nfib is essential for both lung maturation and brain development. *Mol. Cell. Biol.* **25,** 685–698.

Steigemann, P., Molitor, A., Fellert, S., Jackle, H., and Vorbruggen, G. (2004). Heparan sulfate proteoglycan syndecan promotes axonal and myotube guidance by slit/robo signaling. *Curr. Biol.* **14,** 225–230.

Stein, E., and Tessier-Lavigne, M. (2001). Hierarchical organization of guidance receptors: Silencing of netrin attraction by slit through a Robo/DCC receptor complex. *Science* **291,** 1928–1938.

Stoeckli, E. T., and Landmesser, L. T. (1995). Axonin-1, Nr-CAM, and Ng-CAM play different roles in the *in vivo* guidance of chick commissural neurons. *Neuron* **14,** 1165–1179.

Stossel, T. P. (1993). On the crawling of animal cells. *Science* **260,** 1086–94.

Su, M., Merz, D. C., Killeen, M. T., Zhou, Y., Zheng, H., Kramer, J. M., Hedgecock, E. M., and Culotti, J. G. (2000). Regulation of the UNC-5 netrin receptor initiates the first reorientation of migrating distal tip cells in Caenorhabditis elegans. *Development* **127,** 585–594.

Sugimoto, Y., Taniguchi, M., Yagi, T., Akagi, Y., Nojyo, Y., and Tamamaki, N. (2001). Guidance of glial precursor cell migration by secreted cues in the developing optic nerve. *Development* **128,** 3321–3330.

Sugisaki, N., Hirata, T., Naruse, I., Kawakami, A., Kitsukawa, T., and Fujisawa, H. (1996). Positional cues that are strictly localized in the telencephalon induce preferential growth of mitral cell axons. *J. Neurobiol.* **29,** 127–137.

Sundaresan, V., Mambetisaeva, E., Andrews, W., Annan, A., Knoll, B., Tear, G., and Bannister, L. (2004). Dynamic expression patterns of Robo (Robo1 and Robo2) in the developing murine central nervous system. *J. Comp. Neurol.* **468,** 467–481.

Super, H., Martinez, A., Del Rio, J. A., and Soriano, E. (1998). Involvement of distinct pioneer neurons in the formation of layer-specific connections in the hippocampus. *J. Neurosci.* **18,** 4616–4626.

Suto, F., Murakami, Y., Nakamura, F., Goshima, Y., and Fujisawa, H. (2003). Identification and characterization of a novel mouse plexin, plexin-A4. *Mech. Dev.* **120,** 385–396.

Szebenyi, G., Callaway, J. L., Dent, E. W., and Kalil, K. (1998). Interstitial branches develop from active regions of the axon demarcated by the primary growth cone during pausing behaviors. *J. Neurosci.* **18,** 7930–7940.

Takahashi, T., and Strittmatter, S. M. (2001). Plexina1 autoinhibition by the plexin sema domain. *Neuron* **29,** 429–439.

Takahashi, T., Fournier, A., Nakamura, F., Wang, L. H., Murakami, Y., Kalb, R. G., Fujisawa, H., and Strittmatter, S. M. (1999). Plexin-neuropilin-1 complexes form functional semaphorin-3A receptors. *Cell* **99,** 59–69.

Tamagnone, L., Artigiani, S., Chen, H., He, Z., Ming, G. I., Song, H., Chedotal, A., Winberg, M. L., Goodman, C. S., Poo, M., Tessier-Lavigne, M., and Comoglio, P. M. (1999). Plexins

are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. *Cell* **99,** 71–80.

Tanaka, E., and Sabry, J. (1995). Making the connection: Cytoskeletal rearrangements during growth cone guidance. *Cell* **83,** 171–176.

Tanaka, E., Ho, T., and Kirschner, M. W. (1995). The role of microtubule dynamics in growth cone motility and axonal growth. *J. Cell Biol.* **128,** 139–155.

Tanaka, E. M., and Kirschner, M. W. (1991). Microtubule behavior in the growth cones of living neurons during axon elongation. *J. Cell Biol.* **115,** 345–363.

Tanno, T., Takenaka, S., and Tsuyama, S. (2004). Expression and Function of Slit1{alpha}, a Novel Alternative Splicing Product for Slit1. *J. Biochem. (Tokyo)* **136,** 575–581.

Tessier-Lavigne, M., and Goodman, C. S. (1996). The molecular biology of axon guidance. *Science* **274,** 1123–1133.

Tessier-Lavigne, M., Attwell, D., Mobbs, P., and Wilson, M. (1988a). Membrane currents in retinal bipolar cells of the axolotl. *J. Gen. Physiol.* **91,** 49–72.

Tessier-Lavigne, M., Placzek, M., Lumsden, A. G., Dodd, J., and Jessell, T. M. (1988b). Chemotropic guidance of developing axons in the mammalian central nervous system. *Nature* **336,** 775–778.

Thanos, S., and Mey, J. (2001). Development of the visual system of the chick. II. Mechanisms of axonal guidance. *Brain Res. Brain Res. Rev.* **35,** 205–245.

Thanos, S., Puttmann, S., Naskar, R., Rose, K., Langkamp-Flock, M., and Paulus, W. (2004). Potential role of Pax-2 in retinal axon navigation through the chick optic nerve stalk and optic chiasm. *J. Neurobiol.* **59,** 8–23.

Thomas, J. B., Crews, S. T., and Goodman, C. S. (1988). Molecular genetics of the single-minded locus: A gene involved in the development of the Drosophila nervous system. *Cell* **52,** 133–141.

Thompson, I. D., and Morgan, J. E. (1993). The development of retinal ganglion cell decussation patterns in postnatal pigmented and albino ferrets. *Eur. J. Neurosci.* **5,** 341–356.

Tisay, K. T., and Key, B. (1999). The extracellular matrix modulates olfactory neurite outgrowth on ensheathing cells. *J. Neurosci.* **19,** 9890–9899.

Tisay, K. T., Bartlett, P. F., and Key, B. (2000). Primary olfactory axons form ectopic glomeruli in mice lacking p75NTR. *J. Comp. Neurol.* **428,** 656–670.

Toran-Allerand, C. D. (1976). Sex steroids and the development of the newborn mouse hypothalamus and preoptic area *in vitro*: Implications for sexual differentiation. *Brain Res.* **106,** 407–412.

Toran-Allerand, C. D. (1980). Sex steroids and the development of the newborn mouse hypothalamus and preoptic area *in vitro*. II. Morphological correlates and hormonal specificity. *Brain Res.* **189,** 413–427.

Toran-Allerand, C. D. (1984). Gonadal hormones and brain development: Implications for the genesis of sexual differentiation. *Ann. N Y Acad. Sci.* **435,** 101–111.

Toran-Allerand, C. D. (1996). The estrogen/neurotrophin connection during neural development: Is co-localization of estrogen receptors with the neurotrophins and their receptors biologically relevant? *Dev. Neurosci.* **18,** 36–48.

Toran-Allerand, C. D. (2004). Estrogen and the brain: Beyond ER-alpha and ER-beta. *Exp. Gerontol.* **39,** 1579–1586.

Toran-Allerand, C. D., Miranda, R. C., Bentham, W. D., Sohrabji, F., Brown, T. J., Hochberg, R. B., and Mac Lusky, N. J. (1992). Estrogen receptors colocalize with low-affinity nerve growth factor receptors in cholinergic neurons of the basal forebrain. *Proc. Natl. Acad. Sci. USA* **89,** 4668–4672.

Torres, M., Gomez-Pardo, E., and Gruss, P. (1996). Pax2 contributes to inner ear patterning and optic nerve trajectory. *Development* **122,** 3381–3391.

Tran, T. S., and Phelps, P. E. (2000). Axons crossing in the ventral commissure express L1 and GAD65 in the developing rat spinal cord. *Dev. Neurosci.* **22,** 228–236.

Treloar, H., Tomasiewicz, H., Magnuson, T., and Key, B. (1997). The central pathway of primary olfactory axons is abnormal in mice lacking the N-CAM-180 isoform. *J. Neurobiol.* **32,** 643–658.

Treloar, H. B., Nurcombe, V., and Key, B. (1996). Expression of extracellular matrix molecules in the embryonic rat olfactory pathway. *J. Neurobiol.* **31,** 41–55.

Tremblay, G. B., Tremblay, A., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Labrie, F., and Giguere, V. (1997). Cloning, chromosomal localization, and functional analysis of the murine estrogen receptor beta. *Mol. Endocrinol.* **11,** 353–365.

Trousse, F., Marti, E., Gruss, P., Torres, M., and Bovolenta, P. (2001). Control of retinal ganglion cell axon growth: A new role for Sonic hedgehog. *Development* **128,** 3927–3936.

Tsai, H. H., Tessier-Lavigne, M., and Miller, R. H. (2003). Netrin 1 mediates spinal cord oligodendrocyte precursor dispersal. *Development* **130,** 2095–2105.

Van Hartesveldt, C., Moore, B., and Hartman, B. K. (1986). Transient midline raphe glial structure in the developing rat. *J. Comp. Neurol.* **253,** 174–184.

van Horck, F. P., Weinl, C., and Holt, C. E. (2004). Retinal axon guidance: Novel mechanisms for steering. *Curr. Opin. Neurobiol.* **14,** 61–66.

Vassar, R., Chao, S. K., Sitcheran, R., Nunez, J. M., Vosshall, L. B., and Axel, R. (1994). Topographic organization of sensory projections to the olfactory bulb. *Cell* **79,** 981–991.

Vinson, C. R., and Adler, P. N. (1987). Directional non-cell autonomy and the transmission of polarity information by the frizzled gene of Drosophila. *Nature* **329,** 549–551.

von Boxberg, Y., Deiss, S., and Schwarz, U. (1993). Guidance and topographic stabilization of nasal chick retinal axons on target-derived components *in vitro*. *Neuron* **10,** 345–357.

Wadsworth, W. G. (2002). Moving around in a worm: Netrin UNC-6 and circumferential axon guidance in C. elegans. *Trends Neurosci.* **25,** 423–429.

Wadsworth, W. G., Bhatt, H., and Hedgecock, E. M. (1996). Neuroglia and pioneer neurons express UNC-6 to provide global and local netrin cues for guiding migrations in C. elegans. *Neuron* **16,** 35–46.

Wahl, S., Barth, H., Ciossek, T., Aktories, K., and Mueller, B. K. (2000). Ephrin-A5 induces collapse of growth cones by activating Rho and Rho kinase. *J. Cell Biol.* **149,** 263–270.

Walsh, F. S., and Doherty, P. (1997). Neural cell adhesion molecules of the immunoglobulin superfamily: Role in axon growth and guidance. *Annu. Rev. Cell Dev. Biol.* **13,** 425–456.

Walter, J., Henke-Fahle, S., and Bonhoeffer, F. (1987a). Avoidance of posterior tectal membranes by temporal retinal axons. *Development* **101,** 909–913.

Walter, J., Kern-Veits, B., Huf, J., Stolze, B., and Bonhoeffer, F. (1987b). Recognition of position-specific properties of tectal cell membranes by retinal axons *in vitro*. *Development* **101,** 685–696.

Walz, A., McFarlane, S., Brickman, Y. G., Nurcombe, V., Bartlett, P. F., and Holt, C. E. (1997). Essential role of heparan sulfates in axon navigation and targeting in the developing visual system. *Development* **124,** 2421–2430.

Walz, A., Rodriguez, I., and Mombaerts, P. (2002). Aberrant sensory innervation of the olfactory bulb in neuropilin-2 mutant mice. *J. Neurosci.* **22,** 4025–4035.

Wang, H., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., and Tessier-Lavigne, M. (1999a). Netrin-3, a mouse homolog of human NTN2L, is highly expressed in sensory ganglia and shows differential binding to netrin receptors. *J. Neurosci.* **19,** 4938–4947.

Wang, K. H., Brose, K., Arnott, D., Kidd, T., Goodman, C. S., Henzel, W., and Tessier-Lavigne, M. (1999b). Biochemical purification of a mammalian slit protein as a positive regulator of sensory axon elongation and branching. *Cell* **96,** 771–784.

Wang, L., and Denburg, J. L. (1992). A role for proteoglycans in the guidance of a subset of pioneer axons in cultured embryos of the cockroach. *Neuron* **8,** 701–714.

Wang, L., Andersson, S., Warner, M., and Gustafsson, J. A. (2002a). Estrogen actions in the brain. *Sci. STKE* **2002a,** PE29.

Wang, L., Andersson, S., Warner, M., and Gustafsson, J. A. (2003). Estrogen receptor (ER) beta knockout mice reveal a role for ERbeta in migration of cortical neurons in the developing brain. *Proc. Natl. Acad. Sci. USA* **100,** 703–708.

Wang, L. C., Rachel, R. A., Marcus, R. C., and Mason, C. A. (1996). Chemosuppression of retinal axon growth by the mouse optic chiasm. *Neuron* **17,** 849–862.

Wang, S. W., Mu, X., Bowers, W. J., Kim, D. S., Plas, D. J., Crair, M. C., Federoff, H. J., Gan, L., and Klein, W. H. (2002b). Brn3b/Brn3c double knockout mice reveal an unsuspected role for Brn3c in retinal ganglion cell axon outgrowth. *Development* **129,** 467–477.

Wang, Y., Thekdi, N., Smallwood, P. M., Macke, J. P., and Nathans, J. (2002c). Frizzled-3 is required for the development of major fiber tracts in the rostral CNS. *J. Neurosci.* **22,** 8563–73.

Wassle, H. (1982). Morphological types and central projections of ganglion cells in the cat retina. *Prog. Ret. Res.* **1,** 125–152.

White, R., Lees, J. A., Needham, M., Ham, J., and Parker, M. (1987). Structural organization and expression of the mouse estrogen receptor. *Mol. Endocrinol.* **1,** 735–744.

Whitford, K. L., Marillat, V., Stein, E., Goodman, C. S., Tessier-Lavigne, M., Chedotal, A., and Ghosh, A. (2002). Regulation of cortical dendrite development by Slit-Robo interactions. *Neuron* **33,** 47–61.

Whitington, P. M. (1993). Axon guidance factors in invertebrate development. *Pharmacol. Ther.* **58,** 263–299.

Whitington, P. M., Quilkey, C., and Sink, H. (2004). Necessity and redundancy of guidepost cells in the embryonic Drosophila CNS. *Int J. Dev. Neurosci.* **22,** 157–163.

Wilkinson, D. G. (2001). Multiple roles of EPH receptors and ephrins in neural development. *Nat. Rev. Neurosci.* **2,** 155–164.

Williams, S. E., Mann, F., Erskine, L., Sakurai, T., Wei, S., Rossi, D. J., Gale, N. W., Holt, C. E., Mason, C. A., and Henkemeyer, M. (2003). Ephrin-B2 and EphB1 mediate retinal axon divergence at the optic chiasm. *Neuron* **39,** 919–935.

Williams-Hogarth, L. C., Puche, A. C., Torrey, C., Cai, X., Song, I., Kolodkin, A. L., Shipley, M. T., and Ronnett, G. V. (2000). Expression of semaphorins in developing and regenerating olfactory epithelium. *J. Comp. Neurol.* **423,** 565–578.

Williamson, T., Gordon-Weeks, P. R., Schachner, M., and Taylor, J. (1996). Microtubule reorganization is obligatory for growth cone turning. *Proc. Natl. Acad. Sci. USA* **93,** 15221–15226.

Wilson, S. W., Ross, L. S., Parrett, T., and Easter, S. S., Jr. (1990). The development of a simple scaffold of axon tracts in the brain of the embryonic zebrafish, Brachydanio rerio. *Development* **108,** 121–145.

Winberg, M. L., Noordermeer, J. N., Tamagnone, L., Comoglio, P. M., Spriggs, M. K., Tessier-Lavigne, M., and Goodman, C. S. (1998). Plexin A is a neuronal semaphorin receptor that controls axon guidance. *Cell* **95,** 903–916.

Wise, S. P., and Jones, E. G. (1976). The organization and postnatal development of the commissural projection of the rat somatic sensory cortex. *J. Comp. Neurol.* **168,** 313–343.

Wodarz, A., and Nusse, R. (1998). Mechanisms of Wnt signaling in development. *Annu. Rev. Cell Dev. Biol.* **14,** 59–88.

Wu, W., Wong, K., Chen, J., Jiang, Z., Dupuis, S., Wu, J. Y., and Rao, Y. (1999). Directional guidance of neuronal migration in the olfactory system by the protein Slit. *Nature* **400,** 331–336.

Yamada, K. M., Spooner, B. S., and Wessells, N. K. (1970). Axon growth: Roles of microfilaments and microtubules. *Proc. Natl. Acad. Sci. USA* **66,** 1206–1212.

Yamada, K. M., Spooner, B. S., and Wessells, N. K. (1971). Ultrastructure and function of growth cones and axons of cultured nerve cells. *J. Cell Biol.* **49,** 614–635.

Yates, P. A., Roskies, A. L., McLaughlin, T., and O'Leary, D. D. (2001). Topographic-specific axon branching controlled by ephrin-As is the critical event in retinotectal map development. *J. Neurosci.* **21,** 8548–8563.

Yee, K. T., Simon, H. H., Tessier-Lavigne, M., and O'Leary, D. M. (1999). Extension of long leading processes and neuronal migration in the mammalian brain directed by the chemoattractant netrin-1. *Neuron* **24,** 607–622.

Yokoyama, N., Romero, M. I., Cowan, C. A., Galvan, P., Helmbacher, F., Charnay, P., Parada, L. F., and Henkemeyer, M. (2001). Forward signaling mediated by ephrin-B3 prevents contralateral corticospinal axons from recrossing the spinal cord midline. *Neuron* **29,** 85–97.

Yoshida, M., and Colman, D. R. (2000). Glial-defined rhombomere boundaries in developing Xenopus hindbrain. *J. Comp. Neurol.* **424,** 47–57.

Yoshihara, Y., Kawasaki, M., Tamada, A., Fujita, H., Hayashi, H., Kagamiyama, H., and Mori, K. (1997). OCAM: A new member of the neural cell adhesion molecule family related to zone-to-zone projection of olfactory and vomeronasal axons. *J. Neurosci.* **17,** 5830–5842.

Yoshikawa, S., McKinnon, R. D., Kokel, M., and Thomas, J. B. (2003). Wnt-mediated axon guidance via the Drosophila Derailed receptor. *Nature* **422,** 583–588.

Yuan, W., Zhou, L., Chen, J. H., Wu, J. Y., Rao, Y., and Ornitz, D. M. (1999). The mouse SLIT family: Secreted ligands for ROBO expressed in patterns that suggest a role in morphogenesis and axon guidance. *Dev. Biol.* **212,** 290–306.

Zallen, J. A., Yi, B. A., and Bargmann, C. I. (1998). The conserved immunoglobulin superfamily member SAX-3/Robo directs multiple aspects of axon guidance in C. elegans. *Cell* **92,** 217–227.

Zhang, H. Y., and Hoffmann, K. P. (1993). Retinal projections to the pretectum, accessory optic system and superior colliculus in pigmented and albino ferrets. *Eur. J. Neurosci.* **5,** 486–500.

Zhang, J., Richards, L. J., Yarowsky, P., Huang, H., van Zijl, P. C., and Mori, S. (2003). Three-dimensional anatomical characterization of the developing mouse brain by diffusion tensor microimaging. *Neuroimage* **20,** 1639–1648.

Zhang, J. H., Cerretti, D. P., Yu, T., Flanagan, J. G., and Zhou, R. (1996). Detection of ligands in regions anatomically connected to neurons expressing the Eph receptor Bsk: Potential roles in neuron-target interaction. *J. Neurosci.* **16,** 7182–7192.

Zhou, F. Q., and Cohan, C. S. (2001). Growth cone collapse through coincident loss of actin bundles and leading edge actin without actin depolymerization. *J. Cell Biol.* **153,** 1071–1084.

Zhou, F. Q., Waterman-Storer, C. M., and Cohan, C. S. (2002). Focal loss of actin bundles causes microtubule redistribution and growth cone turning. *J. Cell Biol.* **157,** 839–849.

Zou, Y., Stoeckli, E., Chen, H., and Tessier-Lavigne, M. (2000). Squeezing axons out of the gray matter: A role for slit and semaphorin proteins from midline and ventral spinal cord. *Cell* **102,** 363–375.