filopodia project from the body of the growth cone. Between the filopodia lie *lamellipodia*, which are also motile and give the growth cone its characteristic ruffled appearance (Figure 47–6C,D).

Growth cones sense environmental signals through their filopodia: rod-like, actin-rich, membrane-limited structures that are highly motile. Their surface membranes bear receptors for the molecules that serve as directional cues for the axon. Their length—tens of micrometers in some cases—permits the filopodia to sample environments far in advance of the central core of the growth core. Their rapid movements permit them to compile a detailed inventory of the environment, and their flexibility permits them to navigate around cells and other obstacles.

When filopodia encounter signals in the environment, the growth cone is stimulated to advance, retract, or turn. Several motors power these orienting behaviors. One source of power is the movement of actin along myosin, an interaction similar to the one that powers the contraction of skeletal muscle fibers, although the actin and myosin of neurons are different from those in muscle. The assembly of actin monomers into polymeric filaments also contributes a propulsive force for filopodial extension. As the actin filaments are constantly depolymerized at the base of filopodia, the balance of polymerization and depolymerization enables the filopodia to move forward without becoming longer. Depolymerization slows during periods of growth cone advance, leading to greater net forward motion. The movement of membranes along the substrate provides yet another source of forward motion.

The contribution of each type of molecular motor to the advance of the growth cone is likely to vary from one situation to another. Nevertheless, the final step involves the flow of microtubules from the central core of the growth cone into the newly extended tip, thus moving the growth cone ahead and leaving in its wake a new segment of axon. New lamellipodia and filopodia form in the advancing growth cone and the cycle repeats (Figure 47–7).

Accurate pathfinding can occur only if the growth cone's motor action is linked to its sensory function. Therefore, it is crucial that the recognition proteins on the filopodia are signal-inducing receptors and not merely binding moieties that mediate adhesion. The binding of a ligand to its receptor affects growth in diverse ways. In some cases, it engages the cytoskeleton directly, through the intracellular domain of receptors (Figure 47–7). Integrin receptors couple to actin in growth cones when they bind molecules associated with the surface of adjoining cells or the extracellular matrix, thereby influencing motility.

Of equal if not greater importance is the ability of ligand binding to stimulate the formation, accumulation, and even breakdown of soluble intracellular molecules that function as second messengers. These second messengers affect the organization of the cytoskeleton, and in this way regulate the direction and rate of movement of the growth cone.

One important second messenger is calcium. The calcium concentration in growth cones is regulated by the activation of receptors on filopodia, and this affects the organization of the cytoskeleton, which in turn modulates motility. Growth cone motility is optimal within a narrow range of calcium concentrations, called a *set point*. Activation of filopodia on one side of the growth cone leads to a concentration gradient of calcium across the growth cone, providing a possible basis for changes in the direction of growth.

Other second messengers that link receptors and motor molecules include cyclic nucleotides, which modulate the activity of enzymes such as protein kinases, protein phosphatases, and rho-family guanosine triphosphatases (GTPases). In turn, these messengers and enzymes regulate the activity of proteins that regulate the polymerization and depolymerization of actin filaments, thereby promoting or inhibiting axonal extension.

The critical role of intracellular signals in growth cone motility and orientation can be demonstrated using embryonic neurons grown in culture. Application of growth factors to one side of a growth cone activates receptors locally and leads to extension and turning of the growth cone toward the source of the signal. In essence, the factor attracts the growth cone. Yet when cyclic adenosine monophosphate (cAMP) levels in the neuron are decreased, the same stimulus acts as a repellent and the growth cone turns away from the signal (Figure 47–8A). Other repulsive factors can become attractive when levels of the second messenger cyclic guanosine 3′,5′-monophosphate (cGMP) are raised.

Recently, another mechanism for coupling guidance molecules to growth cone behavior has come to light. It was long thought that all neuronal protein synthesis occurs in the cell body, but we now know that growth cones (as well as some dendrites) contain the machinery for protein synthesis, including a subset of messenger RNAs. Initial evidence that these molecules play an important role came from experiments in which axons were severed from their parent cell body. The growth cones continued to advance for a few hours; they could be stimulated to turn toward or away from local depots of guidance molecules, and these behaviors were abolished by inhibitors of protein

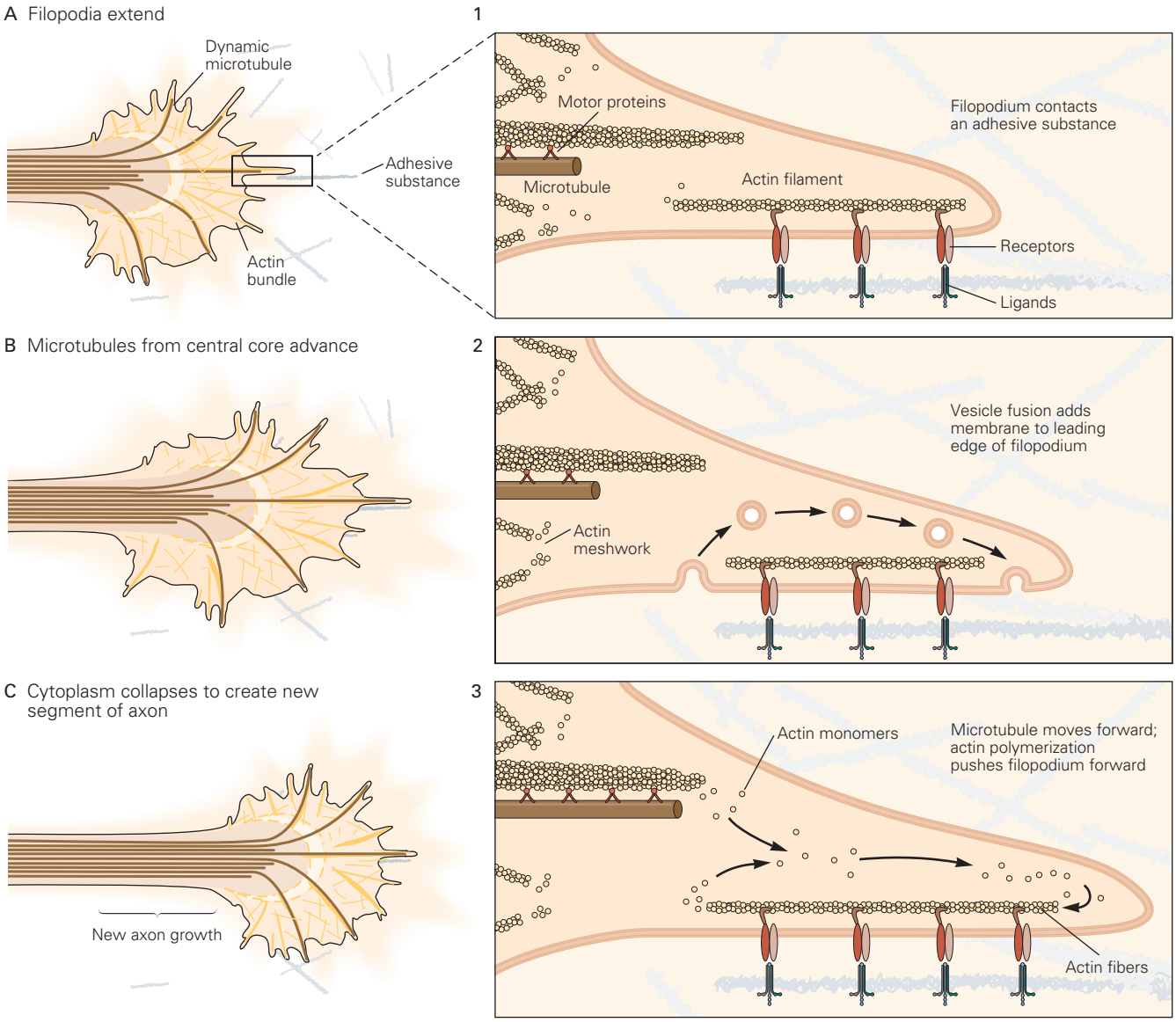


Figure 47–7 The growth cone advances under the control of cellular motors. (Adapted, with permission, from Heidemann 1996. Copyright © 1996 Academic Press Inc.)

A. A filopodium contacts an adhesive cue and contracts, thus pulling the growth cone forward (**1**). Actin filaments assemble at the leading edge of a filopodium and disassemble at the trailing edge, interacting with myosin along the way (**2**). Actin polymerization pushes the filopodium forward (**3**). Force generated by the retrograde flow of actin pushes the filopodium forward. Exocytosis adds membrane to the leading edge of the filopodium and supplies new adhesion receptors to maintain traction. Membrane is recovered at the back of the filopodium. The actin polymer is linked to adhesion molecules on the plasma membrane.

B. The combined action of these motors creates an actin-depleted space that is filled by the advance of microtubules from the central core.

C. Individual microtubules condense to form a thick bundle, and the cytoplasm collapses around them to create a new segment of axonal shaft.

synthesis. The local protein synthesis is regulated by second messengers produced in response to activation of guidance receptors on the growth cones (Figure 47–8). This mechanism leads to synthesis of new motor proteins precisely when and where they are needed. Thus, the growth cone has many strategies and mechanisms for integrating molecular signals to direct the axon in specific directions.

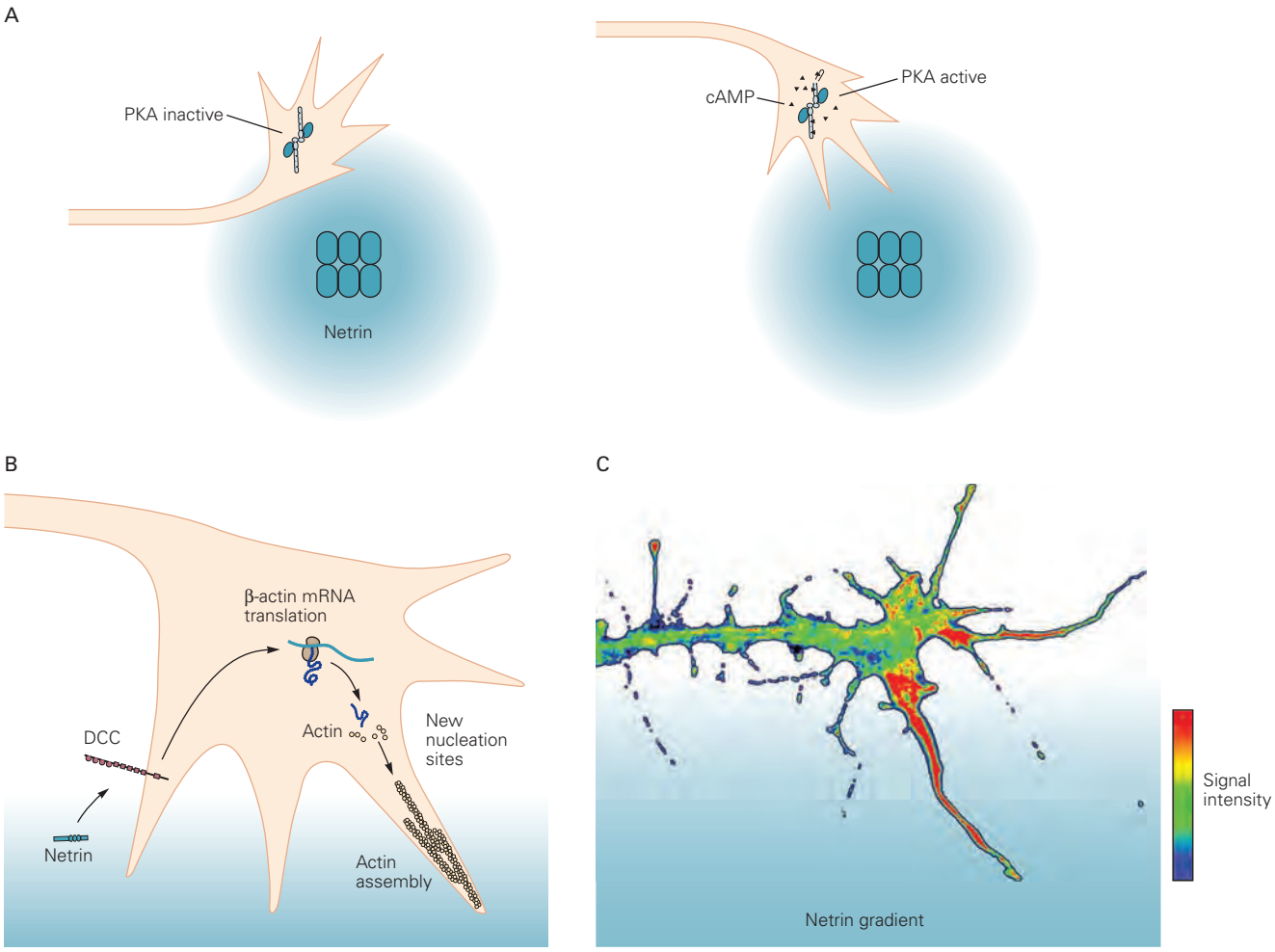


Figure 47–8 Changes in the level of intracellular regulatory proteins can determine whether the same extrinsic cue attracts or repels the growth cone.

A. The state of protein kinase A (**PKA**) activity can alter the growth cone's response to an extracellular orienting factor, in this instance, the protein netrin. When PKA activity and intracellular cyclic adenosine monophosphate (**cAMP**) levels are low, the growth cone is repelled by netrin. When PKA activity is high, the resulting elevation in intracellular cAMP causes the growth cone to be attracted to a local source of netrin. (Adapted, with permission, from Ming et al. 1997.)

B. Netrin activation of growth cone receptors (deleted in colon cancer, **DCC**) leads to local synthesis of actin, which leads to turning.

C. Immunohistochemical analysis of a growth cone showing local synthesis of actin in response to local application of netrin. (Reproduced, with permission, from Christine Holt. Adapted, with permission, from Leung et al. 2006.)

Molecular Cues Guide Axons to Their Targets

For much of the 20th century, a debate raged between advocates of two very different views of how growth cones navigate embryonic terrains to reach their targets. A molecular view of axonal guidance was first articulated at the turn of the 20th century by the physiologist J. N. Langley. But by the 1930s, many eminent biologists, including Paul Weiss, believed that axonal outgrowth was essentially random and that appropriate connections persisted largely because of productive, matching patterns of electrical activity in the axon and its target cell.

In our molecular age, Weiss's ideas may seem simplistic, but they were not unreasonable at the time. In tissue culture, axons grow preferentially along mechanical discontinuities (scratches and bumps on a cover slip), and embryonic nerve trunks often align themselves with solid supports (blood vessels or cartilage). It seemed logical to Weiss that mechanical guidance, called *stereotropism*, could account for axonal patterning. Today, we are quite comfortable with the

idea that electrical signals can be used to change the way current flows in a computer without the need to resolder connections. Likewise, patterns of activity and experience can strengthen or weaken neural connections without requiring the formation of new axonal pathways. Then why not consider that congruent activity, called *resonance* by Weiss, is involved in establishing appropriate connections?

Today, few scientists believe that stereotaxis or resonance is a crucial force in initial patterning of neuronal circuits. The tipping point that shifted opinion in favor of the molecular view was an experiment performed with frogs and other amphibia in the 1940s by Roger Sperry (ironically, a student of Weiss). Sperry manipulated the information carried from the eye to the brain by the axons of retinal ganglion cells. These axons terminate in their target areas—the lateral geniculate body in the thalamus and the superior colliculus (called optic tectum in lower vertebrates) in the midbrain—in such a way that an orderly retinotopic map of the visual field is created.

Because of the optics of the eye, the visual image on the retina is an inversion of the visual field. The retinal ganglion cells reinvert the image by the pattern in which their axons terminate in the optic tectum, the main visual center in the brain of frogs (Figure 47–9A). If the optic nerve is cut, the animal is blinded. In lower vertebrates, cut retinal axons can reestablish projections to the tectum, whereupon vision is restored. This is not the case in mammals, as we will discuss in Chapter 50.

Sperry's key experiment was to sever the optic nerve in a frog and then rotate the eye in its socket by 180° before regeneration of the nerve. Remarkably, the frog exhibited orderly responses to visual input, but the behavior was wrong. When the frog was presented with a fly on the ground, it jumped up, and when offered a fly above its head, it struck downward (Figure 47–9B). Importantly, the animal never learned to correct its mistakes. Sperry suggested—and later verified with anatomical and physiological methods—that the retinal axons had reinnervated their original tectal targets, even though these connections provided the brain with erroneous spatial information that led to aberrant behavior. The inference of these experiments was that recognition between axons and their targets relied on molecular matching rather than functional validation and refinement of random connections.

But Weiss's ideas are by no means obsolete. Indeed, we now recognize that the activity of neural circuits can play a crucial role in shaping connectivity. The current view is that molecular matching predominates during embryonic development and that activity and experience modify circuits after they have formed. In this chapter and the next, we describe the molecular cues that guide the formation of neural connections, and then in Chapter 49, we examine the role of activity and experience in the fine-tuning of synaptic connections.

Sperry's conjecture, often called the *chemospecificity hypothesis*, prompted developmental neurobiologists to search for axonal and synaptic "recognition molecules." Success was limited for the first few decades, in part because these molecules are present in small amounts and on discrete subsets of neurons and there were no effective methods for isolating rare molecules from complex tissues. Eventually advances in biochemical and molecular-biological methods made this task more feasible, and many proteins involved in the guidance of axons to their targets have now been discovered. These proteins typically consist of paired ligands and receptors: The ligands are presented by cells along the pathway an axon follows and the receptors by the growth cone itself.

In the most general terms, guidance cues can be presented on cell surfaces, in the extracellular matrix, or in soluble form. As described above (Figure 47–8), they interact with receptors embedded in the growth cone membrane to promote or inhibit outgrowth of the axon. Most receptors have an extracellular domain that selectively binds the cognate ligand and an intracellular domain that couples to the cytoskeleton, either directly or through intermediates such as second messengers. The ligands can speed or slow growth. Ligands presented to one side of the growth cone can result in local activation or inhibition, leading to turning. In this way, the local distribution of environmental cues determines the pathway of the advancing growth cone.

As a result of these recent discoveries, axon guidance—a process that appeared mysterious years ago—can now be viewed as the orderly consequence of protein–protein interactions that instruct the growth cone to grow, turn, branch, or stop (Figures 47–10, 11). This limited set of instructions is sufficient, when presented with spatial precision, to choreograph growth cone behaviors with exquisite subtlety. Axonal guidance can therefore be explained by describing how and where ligands are presented and how the growth cone integrates this information to generate an orderly response. In the rest of the chapter, we illustrate lessons learned by describing the journeys of two types of axons: those of retinal ganglion neurons and those of a particular class of sensory relay neurons in the spinal cord.

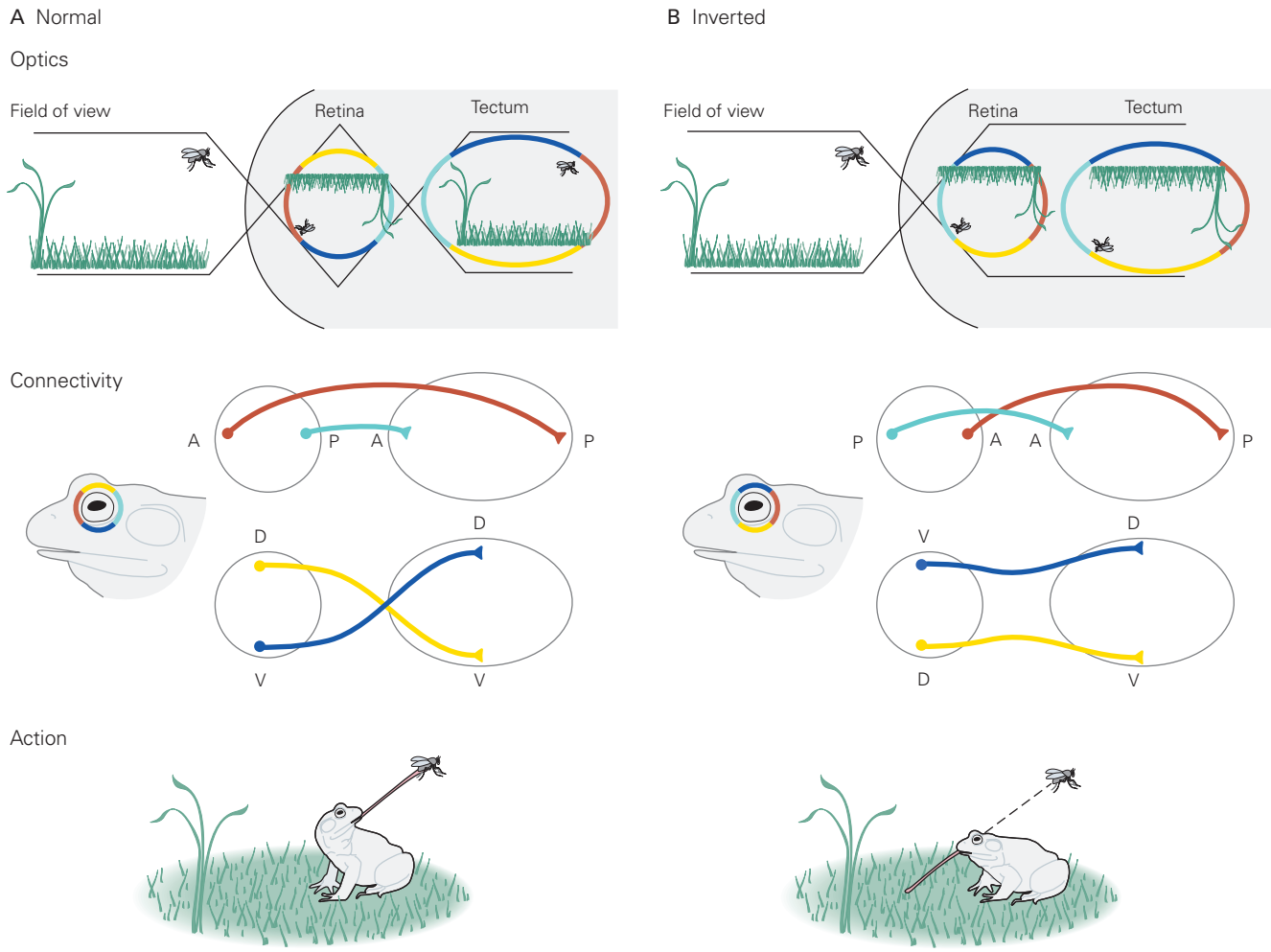


Figure 47–9 Roger Sperry's classical experiments on regeneration in the visual system provided evidence for chemoaffinity in the wiring of connections.

A. In the visual system of the frog, the lens projects an inverted visual image onto the retina and the optic nerve then transfers the image, with an additional inversion, to the optic tectum. The spatial arrangement of retinal inputs to the tectum allows for this transfer. Neurons in the anterior retina project axons to the posterior tectum, while neurons in the posterior retina project to the anterior tectum. Similarly, neurons in the dorsal retina project to the ventral tectum, and neurons in the ventral retina project to the dorsal tectum. As a result, visually guided behaviors (here catching a fly) are accurate. (Abbreviations: **A**, anterior; **D**, dorsal; **P**, posterior; **V**, ventral.)

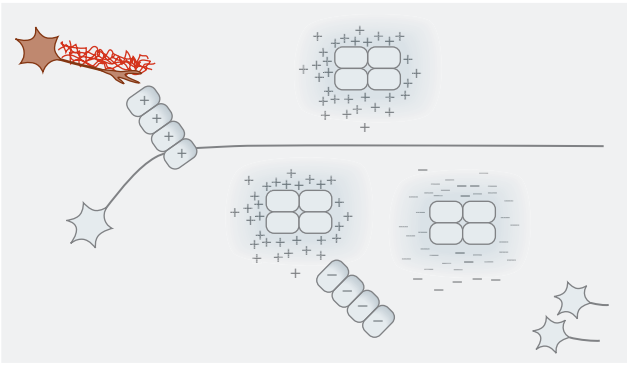
B. If the optic nerve is cut and the eye is surgically rotated in its socket before the nerve regenerates, visually guided behavior is aberrant. When a fly is presented overhead, the frog perceives it as below, and vice versa. The inversion of behavioral reflexes results from the connection of regenerating retinal axons to their original targets, even though these connections now transfer an inverted, inappropriate map of the world into the brain.

The Growth of Retinal Ganglion Axons Is Oriented in a Series of Discrete Steps

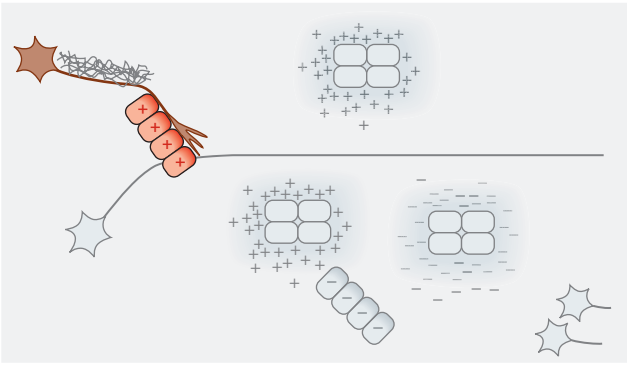
Sperry's experiment implied the existence of axon guidance cues but did not reveal where they were or how they worked. For a time, one prominent view was that recognition occurred mostly at or near the target and that mechanical forces or long-range chemotactic factors sufficed to get axons to the vicinity of the target.

We now know that axons reach distant targets in a series of discrete steps, making frequent decisions at closely spaced intervals along their route. To illustrate this point, we shall trace in greater detail the path that Sperry was trying to understand, that of a retinal axon growing to the optic tectum.

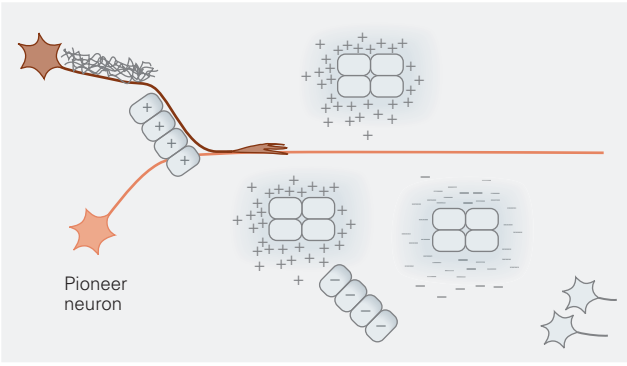
1 Extracellular matrix adhesion



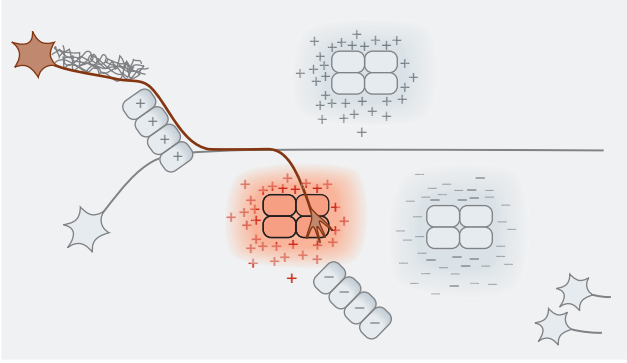
2 Cell surface adhesion



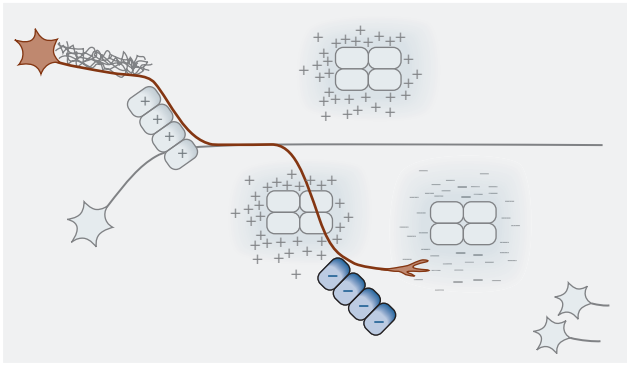
3 Fasciculation



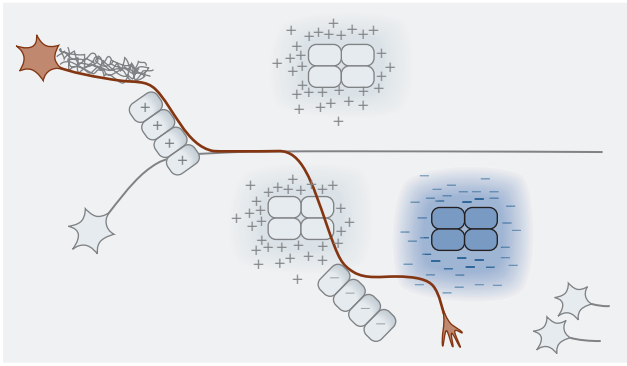
4 Chemoattraction



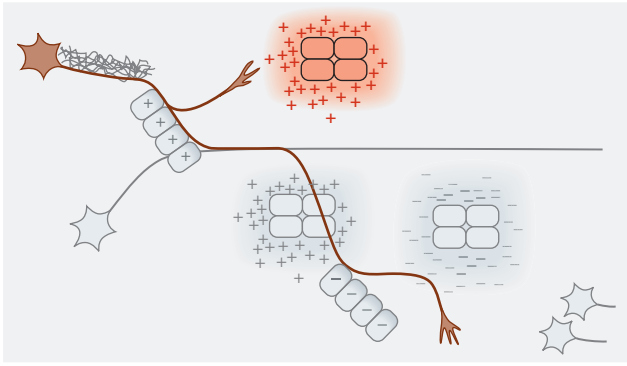
5 Contact inhibition



6 Chemorepulsion



7 Collateral branching



8 Terminal branching

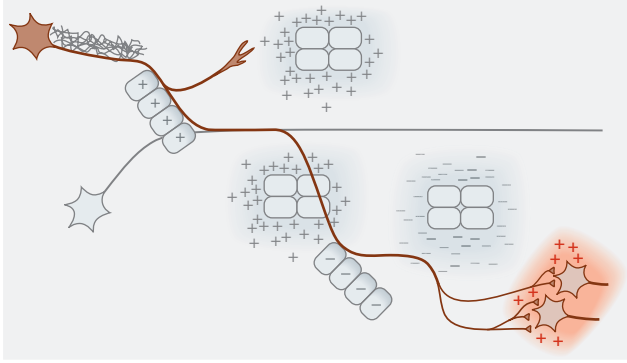
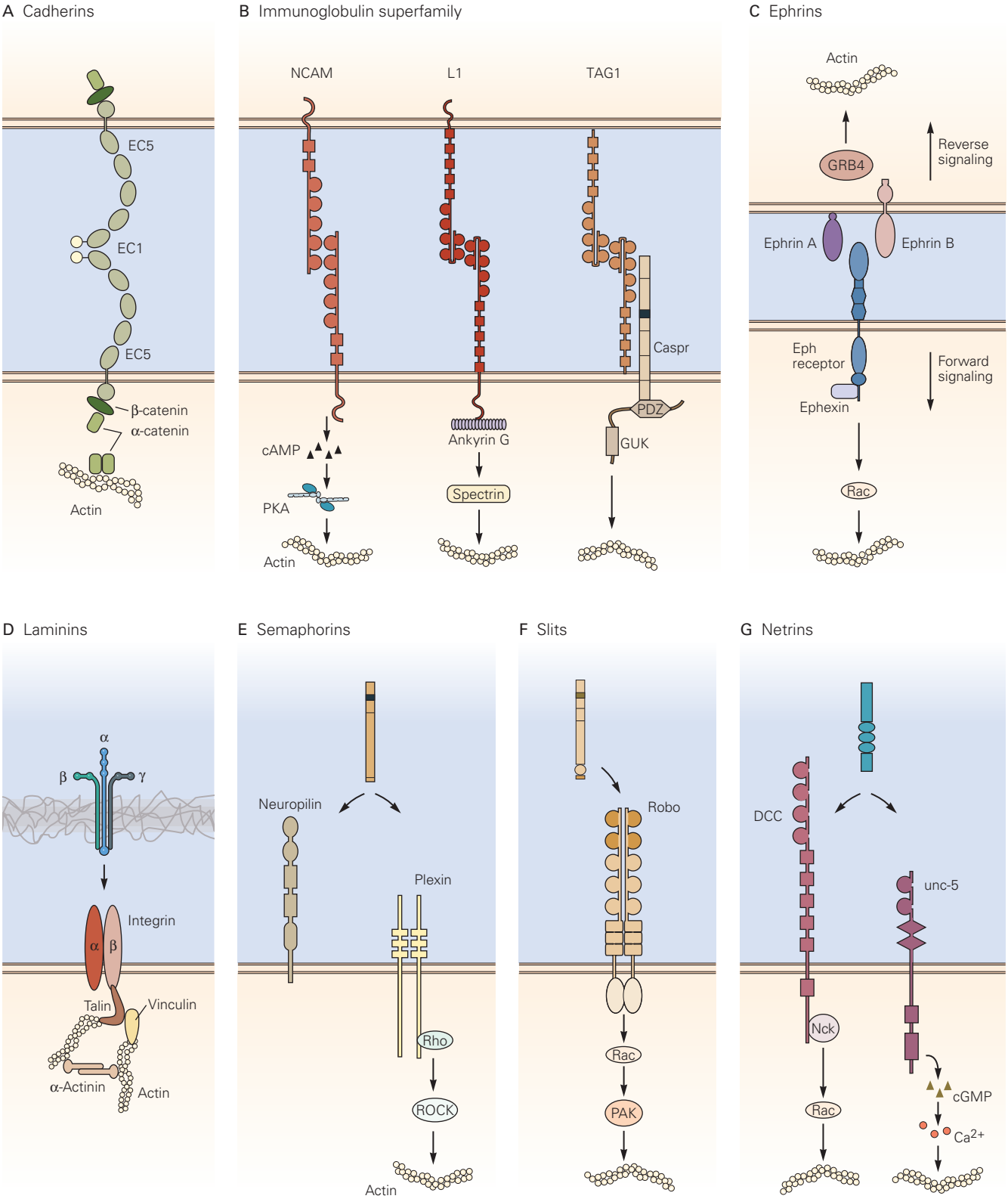


Figure 47–10 **Extracellular cues use a variety of mechanisms to guide growth cones.** The axon can interact with growth-promoting molecules in the extracellular matrix (1). It can interact with adhesive cell-surface molecules on neural cells (2). The growing axon can encounter another axon from a "pioneer" neuron and track along it, a process termed *fasciculation* (3). Soluble chemical signals can attract the growing axon to its cellular source (4). Intermediate target cells that express cell-surface repellent cues can cause the axon to turn away (5). Soluble chemical signals can repel the growing axon (6). Extracellular signals also lead to formation of collaterals from axon shafts (7) or branching of the growing axon (8).


A Cadherins
EC5
EC1
EC5
β-catenin
α-catenin
Actin
B Immunoglobulin superfamily
NCAM
L1
TAG1
Caspr
PDZ
GUK
cAMP
PKA
Actin
Ankyrin G
Spectrin
Actin
C Ephrins
Actin
GRB4
Reverse signaling
Ephrin A
Ephrin B
Eph receptor
Ephexin
Forward signaling
Rac
D Laminins
α
β
γ
Integrin
α
β
Talin
Vinculin
α-Actinin
Actin
E Semaphorins
Neuropilin
Plexin
Rho
ROCK
Actin
F Slits
Robo
Rac
PAK
G Netrins
DCC
unc-5
Nck
Rac
cGMP
Ca2+

Growth Cones Diverge at the Optic Chiasm

The first task of the axon of a retinal ganglion cell is to leave the retina. As it enters the optic fiber layer, it extends along the basal lamina and glial end-feet at the retina's edge. The growth of the axon is oriented from the outset, indicating that it can read directional cues in the environment. As it approaches the center of the retina, it comes under the influence of attractants emanating from the optic nerve head (the junction of the optic nerve with the retina proper), which guide it into the optic stalk. It then follows the optic nerve toward the brain (Figure 47–12).

The first axons to travel this route follow the cells of the optic stalk, the rudiment of the neural tube that connects the retina to the diencephalon from which it arose. These "pioneer" axons then serve as scaffolds for later-arriving axons, which are able to extend accurately simply by following their predecessors (see "fasciculation" in Figure 47–10). Once they reach the optic chiasm, however, the retinal axons must make a choice. Axons that arise from neurons in the nasal hemiretina of each eye cross the chiasm and proceed to the opposite side of the brain, whereas those from the temporal half are deflected as they reach the chiasm and so stay on the same side of the brain (Figure 47–13A).

This divergence in trajectory reflects the differential responses of axons from the nasal and temporal hemiretinas to guidance cues presented by midline chiasm cells. Some retinal axons contact and traverse chiasm cells, whereas others are inhibited by these cells and deflected away, thus remaining on the ipsilateral side. One of the key molecules presented by chiasm cells is a membrane-bound repellent of the ephrin-B family (Figure 47–13B), which also figures in later steps of retinal ganglion cell axon guidance.

The fraction of temporal retinal axons that project ipsilaterally varies among species: few in lower vertebrates, some in rodents, and many in humans. These differences reflect placement of the eyes. In many animals, the eyes point to the sides and monitor different parts of the visual world, so that information from the two eyes need not be combined. In humans, both eyes look forward and sample largely overlapping regions of the visual world, so coordination of visual input is essential.

After crossing the optic chiasm, retinal axons assemble in the optic tract along the ventral surface of the diencephalon. Axons then leave the tract at different points. In most vertebrate species, the tectum of the midbrain (called the superior colliculus in mammals) is the major target of retinal axons, but a small number of axons project to the lateral geniculate nucleus of the thalamus. In humans, however, most axons project to the lateral geniculate, a sizable number reach the

Figure 47–11 (Opposite) Diverse molecular families control the growth and guidance of developing axons.

A. A large family of classical cadherins promote cell and axonal adhesion, primarily through homophilic interactions between cadherin molecules on adjacent neurons. Adhesive interactions are mediated through interactions of the extracellular EC1 domains. Cadherins transduce adhesive interactions though their cytoplasmic interactions with catenins, which link cadherins to the actin cytoskeleton.

B. A diverse array of immunoglobulin superfamily proteins are expressed in the nervous system and mediate adhesive interactions. The three examples shown here, NCAM, L1, and TAG1, can bind both homophilically and heterophilically to promote axon outgrowth and adhesion. These proteins contain both immunoglobulin domains (**circles**) and fibronectin type III domains (**squares**). Homophilic interactions typically involve amino terminal immunoglobulin domains. Different immunoglobulin adhesion molecules interact with the cytoskeleton via diverse cytoplasmic mediators, only a few of which are shown here.

C. Different ephrin proteins bind to Eph class tyrosine kinase receptors. Class A ephrins are linked to the surface membrane through a glycosyl phosphatidylinositol tether, whereas class B ephrins are transmembrane proteins. Class A ephrins typically bind class A Eph kinases, and class B ephrins typically bind class B Eph kinases. Forward Eph signaling usually elicits repellant or inhibitory responses in receptive cells, whereas reverse ephrin signaling can elicit adhesive or inhibitory responses. Ephrin-Eph signaling involves many different cytoplasmic mediators.

D. Laminin proteins are components of the extracellular matrix and promote cell adhesion and axon extension through interactions with integrin receptors. Integrins mediate adhesion and axon growth through interactions with the cytoskeleton via many intermediary proteins.

E. Semaphorin proteins can promote or inhibit axonal growth through interaction with a diverse array of plexin and neuropilin receptors, which transduce signals via Rho class GTPases and downstream kinases.

F. Slit proteins typically mediate repellant responses through interaction with Robo class receptors, which influence axonal growth via intermediary GTPases such as Rac.

G. The secreted or extracellular matrix–associated netrin proteins mediate both chemoattractant and chemorepellent responses. Attractant responses are mediated through interaction with **DCC** (deleted in colorectal cancer) receptors, whereas repellent responses involve interactions with DCC and unc-5 coreceptors. Netrin receptors signal via GTPases and cyclic guanosine monophosphate (**cGMP**) cascades.

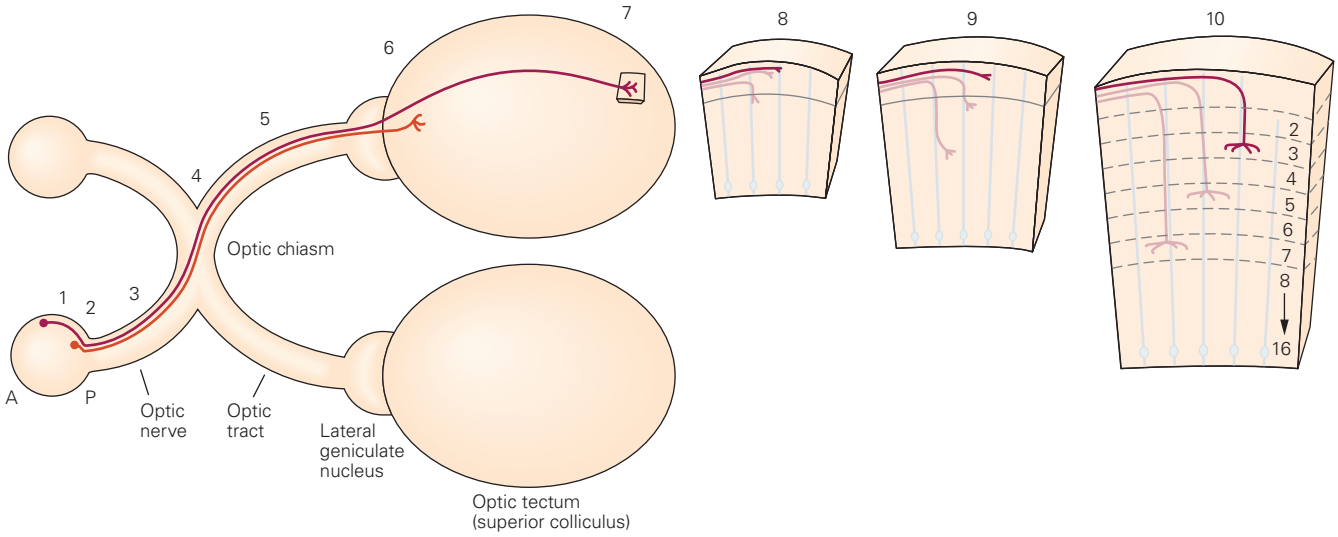


Figure 47–12 The axons of retinal ganglion cells grow to the optic tectum in discrete steps. Two neurons that carry information from the nasal half of the retina are shown. The axon of one crosses the optic chiasm to reach the contralateral optic tectum. The axon of the other also crosses the optic chiasm but projects to the lateral geniculate nucleus. The numbers indicate important landmarks on the axon's journey. The growing axon is directed toward the optic nerve head (the junction of the nerve with the retina) (**1**), enters into the optic nerve (**2**), extends through the optic nerve (**3**), swerves to remain ipsilateral (not shown) or crosses to the contralateral side at the optic chiasm (**4**), extends through the optic tract (**5**), enters into the optic tectum or lateral geniculate nucleus (not shown) (**6**), navigates to an appropriate rostrocaudal and dorsoventral position on the tectum (**7**), turns to enter the neuropil (descends in chicks as shown here; ascends in mammals) (**8**), stops at an appropriate layer where a rudimentary terminal arbor is formed (**9**), and finally is remodeled (**10**). (Abbreviations: **A**, anterior; **P**, posterior).

colliculus, and small numbers project to the pulvinar, superchiasmatic nucleus, and pretectal nuclei. Within these targets, different retinal axons project to different regions. As Sperry showed, the retinal axons form a precise retinotopic map on the tectal surface. Similar maps form in other areas innervated by retinal axons such as the lateral geniculate nucleus.

Having reached an appropriate position within the tectum, retinal axons need to find an appropriate synaptic partner. To achieve this last leg of their journey, retinal axons turn and dive into the tectal neuropil (Figure 47–12), descending (or, in mammals, ascending) along the surface of radial glial cells, which provide a scaffold for radial axonal growth. Although radial glial cells span the entire extent of the neuroepithelium, each retinal axon confines its synaptic terminals to a single layer. The dendrites of many postsynaptic cells extend through multiple layers and form synapses along their entire length, but retinal inputs are restricted to a small fraction of the target neuron's dendritic tree. These organizational features imply that layer-specific cues arrest axonal elongation and trigger arborization.

The problem of long-distance axon navigation is therefore solved by dividing the journey into short segments in which intermediate targets guide the axons along the path to their final targets. Some intermediate targets, such as the optic chiasm, are "decision" regions where axons diverge.

Reliance on intermediate targets is an effective solution to the problem of long-distance axonal navigation but is not the only one. In some cases, the first axons reach their targets when the embryo is small and the distance to be covered is short. These "pioneer" axons respond to molecular cues embedded in cells or the extracellular matrix along their way. The first axons to exit the retina fall within this class. Axons that appear later, when distances are longer and obstacles more numerous, can reach their targets by following the pioneers. Yet another guidance mechanism is a molecular gradient. Indeed, as we will see, gradients of cell-surface molecules in the tectum inform axons about their proper termination zone.

Gradients of Ephrins Provide Inhibitory Signals in the Brain

So far, we have seen how retinal axons reach the tectum by responding to a series of discrete directional cues. However, these choices during growth do not account