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48

Formation and Elimination of Synapses

So far, we have examined three stages in the development of the mammalian nervous system: the formation and patterning of the neural tube, the generation and differentiation of neurons and glia, and the growth and guidance of axons. One additional step must occur before the brain becomes functional: the formation of synapses. Only when synapses are formed and functional can the brain go about the business of processing information.

Three key processes drive synapse formation. First, axons make choices among many potential postsynaptic partners. By forming synaptic connections only on particular target cells, neurons assemble functional circuits that can process information. In many cases, synapses are even formed at specific sites on the postsynaptic cell; some types of axons form synapses on dendrites, others on cell bodies, and yet others on axons or nerve terminals. Although cellular and subcellular specificity are evident throughout the brain, the general features of synapse formation can be illustrated with a few well-studied examples.

Second, after cell–cell contacts have formed, the portion of the axon that contacts the target cell differentiates into a presynaptic nerve terminal, and the domain of the target cell contacted by the axon differentiates into a specialized postsynaptic apparatus. Precise coordination of pre- and postsynaptic differentiation depends on interactions between the axon and its target cell. Much of what we know about these interactions comes from studies of the neuromuscular junction, the synapse between motor neurons and skeletal muscle fibers. The simplicity of this synapse made it a favorable system to probe the structural and electrophysiological principles of chemical synapses (Chapter 12), and this simplicity has also helped in the analysis of developing synapses. We will use the neuromuscular synapse to illustrate key features of

synaptic development and then apply insights from this peripheral synapse to examine synapses that form in the brain.

Finally, once formed, synapses mature, often undergoing major rearrangements. One striking aspect of the rearrangement is that as some synapses grow and strengthen, many others are eliminated. Like neuronal cell death (Chapter 46), synapse elimination at first glance is a puzzling and seemingly wasteful step in neural development. It is increasingly clear, however, that it plays a key role in refining initial patterns of connectivity. We will discuss the main features of synaptic rearrangement at the neuromuscular junction, where it has been studied intensively, as well as at synapses between neurons, where it also is prominent.

Synapse formation stands at an interesting crossroads in the sequence of events that assemble the nervous system. The initial steps in this process appear to be largely "hardwired" by molecular programs. However, as soon as synapses form, the nervous system begins to function, and the activity of neural circuits plays a critical role in subsequent development. Indeed, the information-processing capacity of the nervous system is refined through its use, most dramatically in early postnatal life but also into adulthood. In this sense, the nervous system continues to develop throughout life. We will consider this interplay of molecular programs and neural activity as we describe synapse formation and rearrangement. This discussion will be a useful prelude to Chapter 49, in which we discuss how genes and the environment—nature and nurture—interact to customize nervous systems early in postnatal life.

Neurons Recognize Specific Synaptic Targets

Once axons reach their designated target areas, they must choose appropriate synaptic partners from the many potential targets within easy reach. Although synapse formation is a highly selective process at both cellular and subcellular levels, few of the molecules that confer synaptic specificity have been identified.

The specificity of synaptic connections is particularly evident when intertwined axons select subsets of target cells. In these cases, axon guidance and selective synapse formation can be distinguished. The first report of such specificity came more than 100 years ago when J. N. Langley, studying the autonomic nervous system, proposed the first version of a chemospecificity hypothesis (see Chapter 46). Langley observed that autonomic preganglionic neurons are generated at distinct rostrocaudal levels of the spinal cord. Their axons enter sympathetic ganglia together but form synapses with different postsynaptic neurons that innervate distinct targets. Using behavioral assays as a guide, Langley inferred that the axons of preganglionic neurons located in the rostral spinal cord form synapses on ganglion neurons that project their axons to relatively rostral targets such as the eye, whereas neurons that derive from more caudal regions of the spinal cord synapse on ganglion neurons that project to caudal targets such as the ear (Figure 48–1A). He then showed that similar patterns were reestablished after the preganglionic axons were severed and allowed to regenerate, leading him to postulate that some sort of molecular recognition was responsible (Figure 48–1B).

Electrophysiological studies later confirmed Langley's intuition about the specificity of synaptic connections in these ganglia. Moreover, this selectivity is apparent from early stages of innervation, even though specific types of postsynaptic neurons are interspersed within the ganglion. The reestablishment of selectivity in adults after nerve damage shows that specificity does not emerge through peculiarities of embryonic timing or neuronal positioning.

Recognition Molecules Promote Selective Synapse Formation in the Visual System

To illustrate the idea of target specificity in more detail, we will first consider retinal ganglion cells. These neurons differ in their response properties—some ganglion neurons respond to increases in light level (ON cells), others to decreases (OFF cells), others to moving objects, and still others to light of a particular color. The axons of all ganglion cells run through the optic nerve, forming parallel axonal pathways from the retina to the brain.

The response properties of each class of ganglion cell depend on the synaptic inputs they receive from amacrine and bipolar interneurons, which in turn receive synapses from light-sensitive photoreceptors. All of the synapses from bipolar and amacrine cells onto ganglion cell dendrites occur in a narrow zone of the retina called the inner plexiform layer. Axons and dendrites therefore have the daunting task of recognizing their correct partners within a large crowd of inappropriate bystanders.

One important contributor to synaptic matchmaking in the inner plexiform layer is its division into sublayers. The processes of each amacrine and bipolar cell type, as well as the dendrites of each functionally distinct ganglion cell type, branch and synapse in just one or a few of approximately 10 sublayers. For example, the dendrites of ON and OFF cells are restricted to inner and outer portions of the plexiform layer,

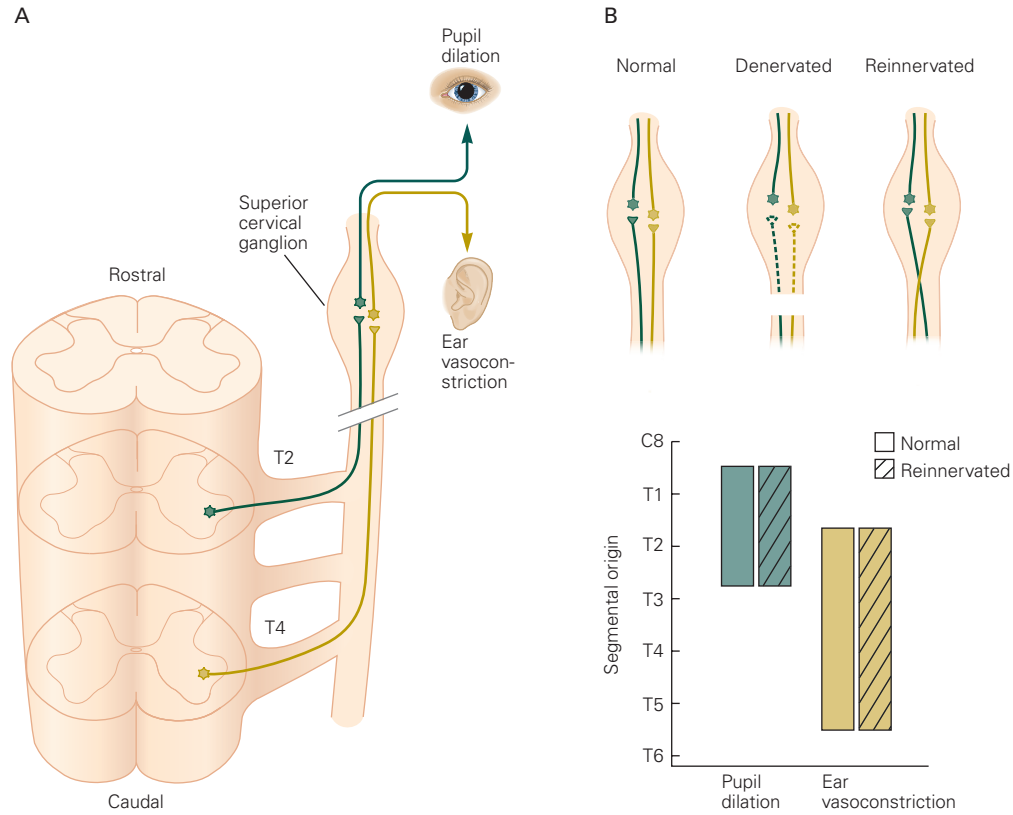

Figure 48–1 Preganglionic motor neurons regenerate selective connections with their sympathetic neuronal targets.

A. Preganglionic motor neurons arise from different levels of the thoracic spinal cord. Axons that arise from rostrally located thoracic neurons innervate superior cervical ganglion neurons that project to rostral targets, including the intrinsic eye muscles. Axons that arise from neurons at caudal levels of the thoracic spinal cord innervate ganglion neurons that project to more caudal targets, such as the blood vessels of the ear. These two classes of ganglion neurons are intermingled in the ganglion, which suggested to J. N. Langley that preganglionic axons from different thoracic levels selectively form synapses with ganglion neurons that terminate in specific peripheral targets.

B. After nerve damage in adults, similar segment-specific patterns of connectivity form during reinnervation, supporting the notion that synapse formation is selective. (Adapted from Njå and Purves 1977.)

respectively, and therefore receive synapses from different interneurons; particular types of ON and OFF cells have narrower restrictions within these zones (Figure 48–2). This layer-specific arborization of pre- and postsynaptic processes restricts the choice of synaptic partners to which they have ready access. Similar lamina-specific connections are found in many other regions of the brain and spinal cord. For example, in the cerebral cortex, distinct populations of axons confine their dendritic arbors and synapses to just one or two of the six main layers.

Laminar specificity does not, however, completely account for the wiring of the retina. As the number of retinal cell types—currently estimated at around 130 in mice—greatly exceeds the number of plexiform sublayers, the processes of many cell types arborize within each sublayer. Anatomical and physiological studies have shown that connectivity is specific even within individual sublayers. Moreover, patterns of connectivity appear to be largely, although not entirely, "hardwired," occurring before visual experience has a chance to affect circuitry. Thus, there must be molecules that restrict axons and dendrites to specific sublayers, as well as molecules that distinguish synaptic partners within a sublayer.

One clue to the basis of both laminar and intralaminar synaptic specificity in the retina comes from the finding that specific types of interneurons and ganglion neurons express different classes of recognition molecules of the immunoglobulin and cadherin families (Chapter 47). Thus, the processes of cells that express a particular recognition molecule are confined

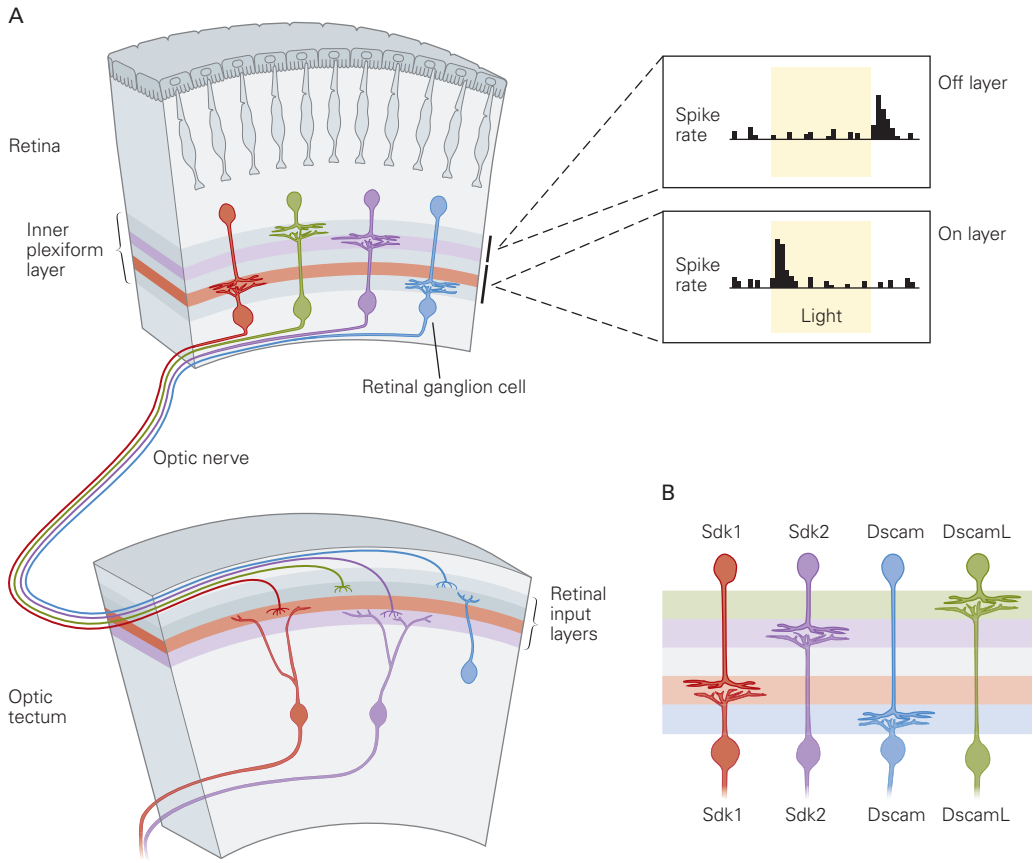

Figure 48–2 Retinal ganglion neurons form layer-specific synapses. (Reproduced, with permission, from Sanes and Yamagata 2009.)

A. The dendrites of retinal ganglion neurons receive input from the processes of retinal interneurons (amacrine and bipolar cells) in the inner plexiform layer, which is subdivided into at least 10 sublaminae. Specific subsets of interneurons and ganglion cells often arborize and synapse in just one layer. These lamina-specific connections determine which aspects of visual stimuli (their onset or offset) activate each type of retinal ganglion cell. The responses of OFF and ON retinal ganglion cells are shown on the right.

B. Immunoglobulin superfamily adhesion molecules (Sdk1, Sdk2, Dscam, and DscamL) are expressed by different subsets of amacrine and retinal ganglion neurons in the developing chick embryo. Amacrine neurons that express one of these four proteins form synapses with retinal ganglion cells that express the same protein. Manipulating Sdk or Dscam expression alters these patterns of lamina-specific arborization.

to one or a few plexiform sublayers (Figure 48–2B). Many of these proteins promote homophilic interactions; that is, they bind to the same protein on other cell surfaces. The roles of several recognition molecules have now been assessed in chick and mouse retina, either by removing them during development or by implanting them into neurons that do not normally express them. Results of these so-called "loss-of-function" and "gain-of-function" experiments hint at the existence of a complex code of recognition molecules that promotes specific connectivity within a target region. In mice, for example, two cadherins direct bipolar interneurons to appropriate sublayers, while Sidekick 2, a member of the immunoglobulin superfamily, is required for interneurons to choose among ganglion cells with dendrites in one particular sublayer.

Sensory Receptors Promote Targeting of Olfactory Neurons

A different type of specificity is evident in the olfactory system. Each olfactory sensory neuron in the nasal epithelium expresses just one of approximately 1,000 types of odorant receptors. Neurons expressing one receptor are randomly distributed across a large sector of the epithelium, yet all of their axons converge on the dendrites of just a few target neurons in the olfactory

bulb, forming synapse-rich glomeruli (Figure 48–3A). When an individual olfactory receptor is deleted, the axons that normally express the receptor reach the olfactory bulb but fail to converge into specific glomeruli or to terminate on the appropriate postsynaptic cells (Figure 48–3B). Conversely, when neurons are forced to express a different odorant receptor, their axons form glomeruli at a different position within the olfactory bulb (Figure 48–3C).

Together, these experiments suggest that olfactory receptors not only determine a neuron's responsiveness to specific odorants but also help the axon to form appropriate synapses on target neurons. Initially, it was suspected that specific olfactory receptors served not only as odor detectors but also as recognition molecules. More recent studies provide evidence for a different mechanism: that second messengers generated from activation of the olfactory receptors influence the expression of recognition molecules that match olfactory axons with appropriate targets in the olfactory bulb.

The matching occurs in two steps. First, intrinsic differences in the abilities of olfactory receptors to stimulate formation of the second messenger cyclic adenosine monophosphate lead to differential expression of guidance molecules in embryos, generating a coarse matching of olfactory neurons and olfactory bulb targets along the anterior-posterior axis. Second, selective expression of recognition molecules by four groups of olfactory sensory neurons targets them to corresponding domains along the dorsoventral axis of the olfactory bulb.

Thus, an early phase of molecular recognition generates a coarse map of nose-to-brain connectivity by activity-independent mechanisms (Figure 48–4A). Then, postnatally, odorant receptors are activated by odorants, and because of developmental changes in

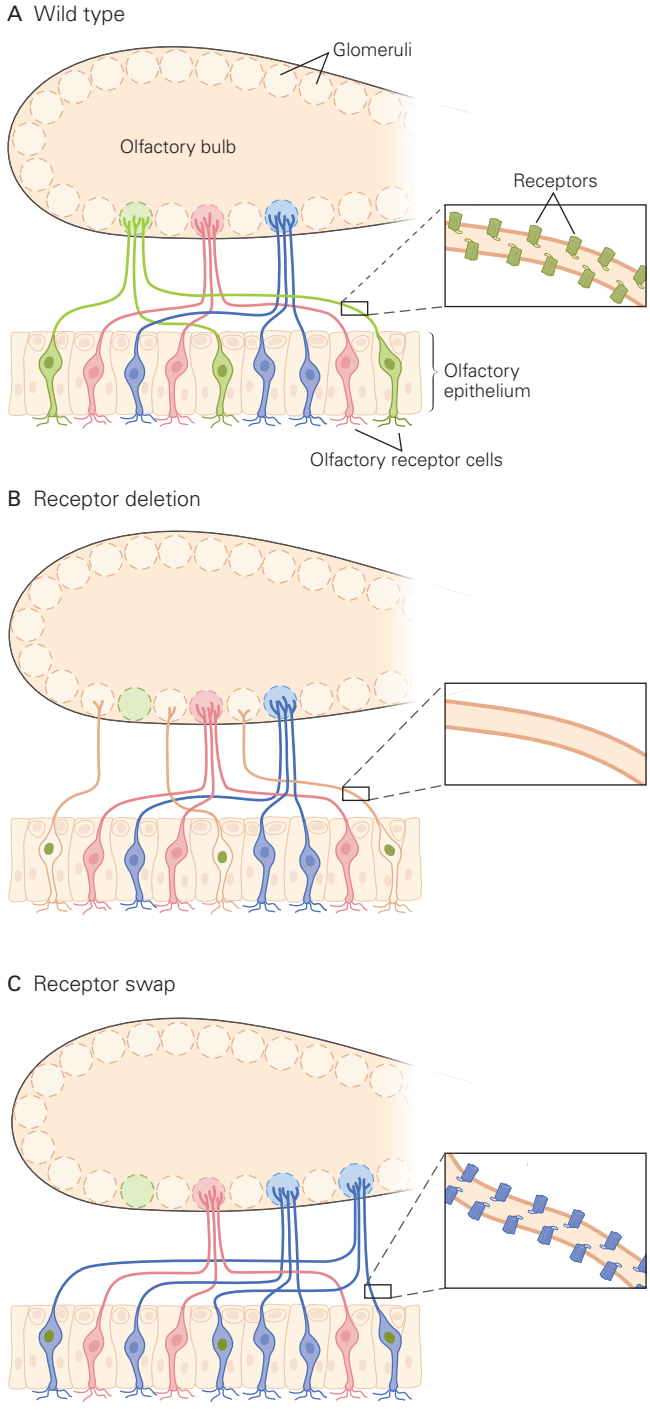

Figure 48–3 (Right) Odorant receptors influence the targeting of sensory axons to discrete glomeruli in the olfactory bulb. (Adapted, with permission, from Sanes and Yamagata 2009.)

A. Each olfactory receptor neuron expresses one of approximately 1,000 possible odorant receptors. Neurons expressing the same receptor are distributed sparsely throughout the olfactory epithelium of the nose. The axons of these neurons form synapses with target neurons in a single glomerulus in the olfactory bulb.

B. In mouse mutants in which an odorant receptor gene has been deleted, the sensory neurons that would have expressed the gene send their axons to other glomeruli, in part because these neurons now express other receptors.

C. When one odorant receptor gene replaces another in a set of sensory neurons, their axons project improperly.

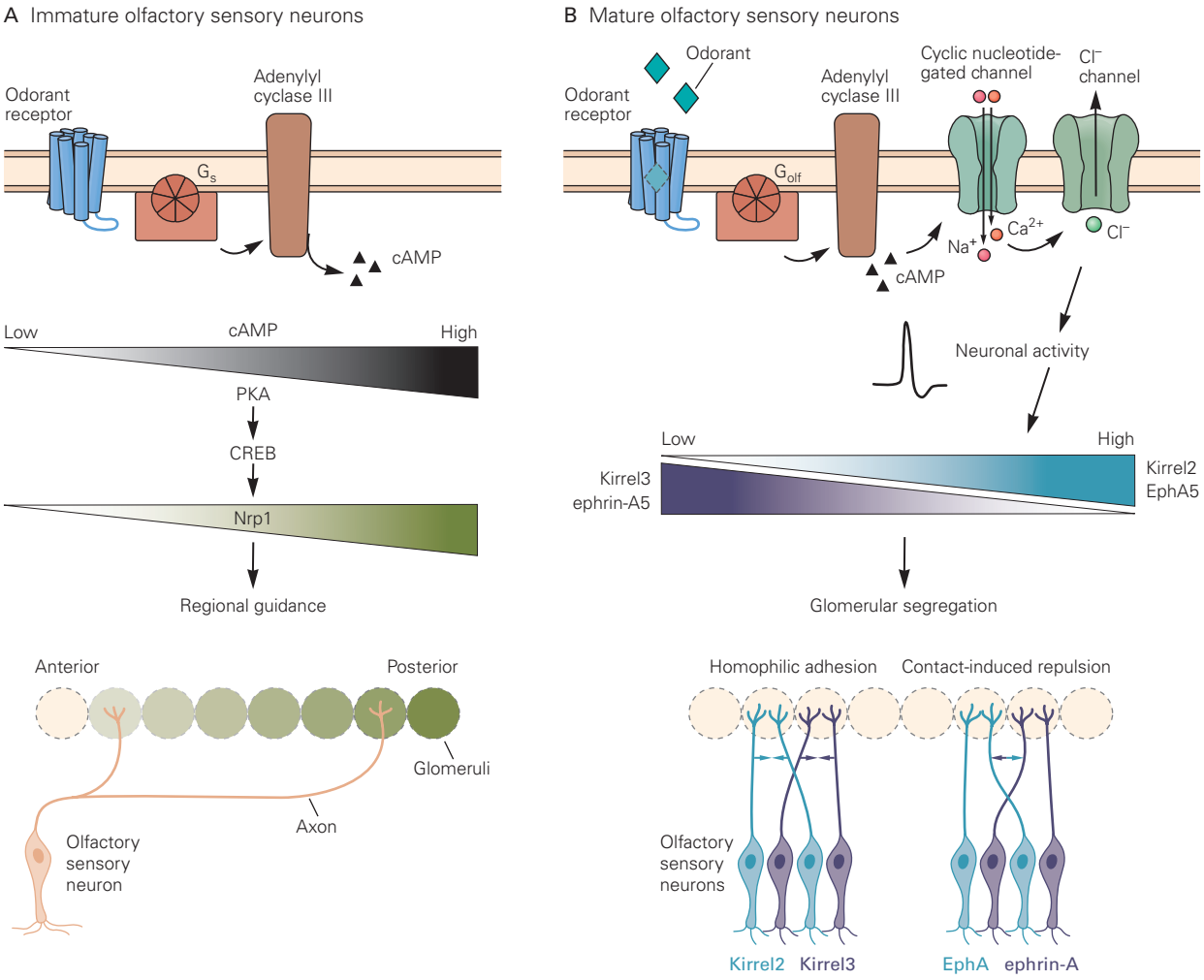

Figure 48–4 Odorant receptors promote specific connections in the olfactory bulb by controlling expression of guidance and recognition molecules. Activation of olfactory receptors in olfactory sensory neurons leads to activation of adenylyl cyclase and production of the second messenger cyclic adenosine monophosphate (**cAMP**).

A. Prenatally, prior to olfaction, the receptors are spontaneously active. Different receptor types exhibit different levels of spontaneous activity and therefore generate different levels of cAMP, which in turn induce distinct, graded levels of axon guidance molecules such as neuropilins and semaphorins. These guidance molecules mediate interactions among axons that guide them to appropriate regions of the olfactory bulb. (Abbreviations: **CREB**, cAMP response element-binding protein; **Nrp1**, neuropilin1; **PKA**, protein kinase A.)

B. Postnatally, olfactory receptors are activated by odorant molecules. This olfactory activity also generates distinct levels of cAMP in each type of odorant receptor neuron, but now the second messenger acts through ion channels to induce new sets of guidance molecules such as kirrels and ephrins. These molecules mediate interactions that segregate axonal terminals into glomeruli. Thus, successive phases of receptor activity, the first spontaneous and the second evoked by odorants, act together to map olfactory sensory axons of different types onto different glomeruli.

intracellular signaling, this activation leads to induction of a second set of recognition molecules. These molecules lead to convergence of axons onto glomeruli, thus refining the projection by an activity-dependent mechanism (Figure 48–4B). Segregation of axons first to particular regions and then to particular glomeruli occurs via both adhesive and repulsive interactions.

Different Synaptic Inputs Are Directed to Discrete Domains of the Postsynaptic Cell

Nerve terminals not only discriminate among candidate targets but also terminate on a specific portion of the target neuron. In the cerebral cortex and hippocampus, for example, axons arriving in layered structures

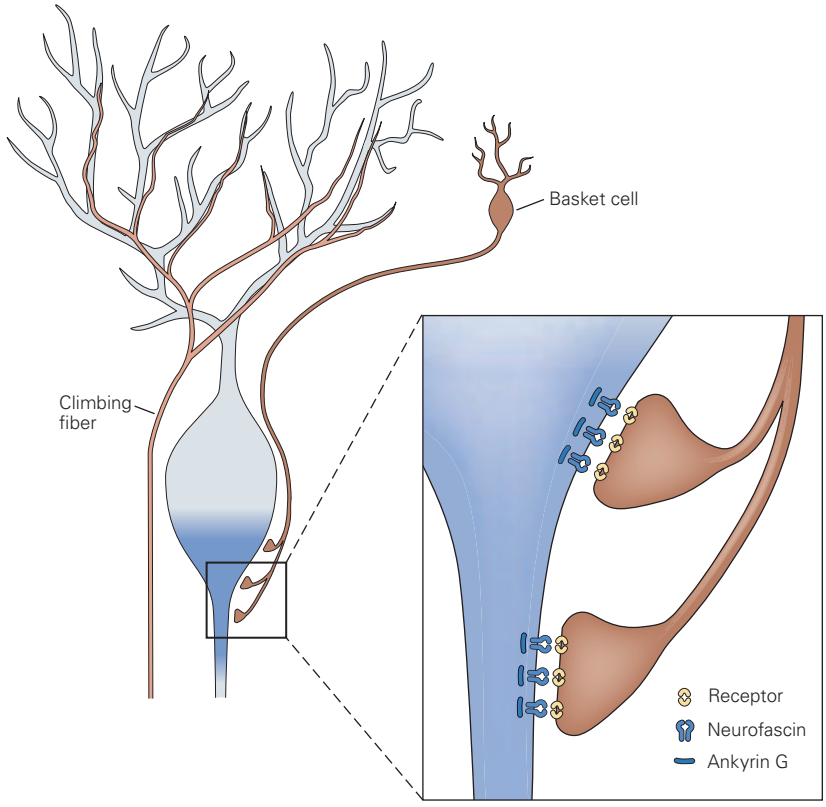

Figure 48–5 The axons of inhibitory interneurons in the cerebellum terminate on a distinct region of the cerebellar Purkinje cell. Many neurons form synapses on cerebellar Purkinje neurons, each selecting a distinct domain on the Purkinje cell. The axons of inhibitory basket cells form most of their synapses on the axon hillock and initial segment. Basket cells select these domains by recognizing neurofascin, a cell surface immunoglobulin superfamily adhesion molecule that is anchored to the initial segment of the axon by ankyrin G. When the localization of neurofascin is perturbed, basket cell axons fail to restrict synapse formation to the initial segment. (Adapted from Huang 2006.)

often confine their terminals to one layer, even if the dendritic tree of the postsynaptic cell traverses numerous layers. In the cerebellum, the axons of different types of neurons terminate on distinct domains of the Purkinje neurons. Granule cell axons contact distal dendritic spines, climbing fiber axons contact proximal dendritic shafts, and basket cell axons contact the axon hillock and initial segment (Figure 48–5).

Such specificity presumably relies on molecular cues on the postsynaptic cell surface. For Purkinje neurons of the cerebellum, one such cue is neurofascin, an adhesion molecule of the immunoglobulin superfamily. Neurofascin is present at high levels on the axonal initial segment, thus directing basket cells to form axons selectively on this axonal domain. Adhesion molecules can therefore also serve as recognition molecules for particular domains of a neuron. Since individual neurons can form synapses with several classes of pre- and postsynaptic cells, it follows that each neuronal subtype must express a variety of synaptic recognition molecules.

Neural Activity Sharpens Synaptic Specificity

So far, we have emphasized the role of recognition molecules in the initial formation of synapses. Once synapses form, however, neural activity within the circuit plays a critical role in refining synaptic patterns. For example, as described above, guidance of olfactory neurons to the olfactory bulb includes an initial activity-independent crude mapping followed by an activity-dependent phase in which the projection is refined.

A similar biphasic pattern has been studied in detail in the visual system. Retinal ganglion cells project to the optic tectum (superior colliculus), where interactions between ephrins and Eph kinases result in formation of a crude retinotopic map of retinal axons on the tectal surface (Chapter 47). Activity-dependent processes then sculpt the axonal arbors of retinal ganglion cells. The axons initially form broad diffuse arbors, which gradually become denser but more focused, sharpening the tectal map (Figure 48–6). This refinement is inhibited when the activity of synapses is blocked. The molecular mechanisms of this activity-dependent refinement are largely unknown. As in the olfactory system, an attractive idea is that the level and pattern of neuronal activity regulate the expression of recognition molecules.

These examples from the olfactory and visual systems illustrate a widespread phenomenon: Molecular cues initially control synapse specificity, but once the circuit begins to function, specificity is sharpened

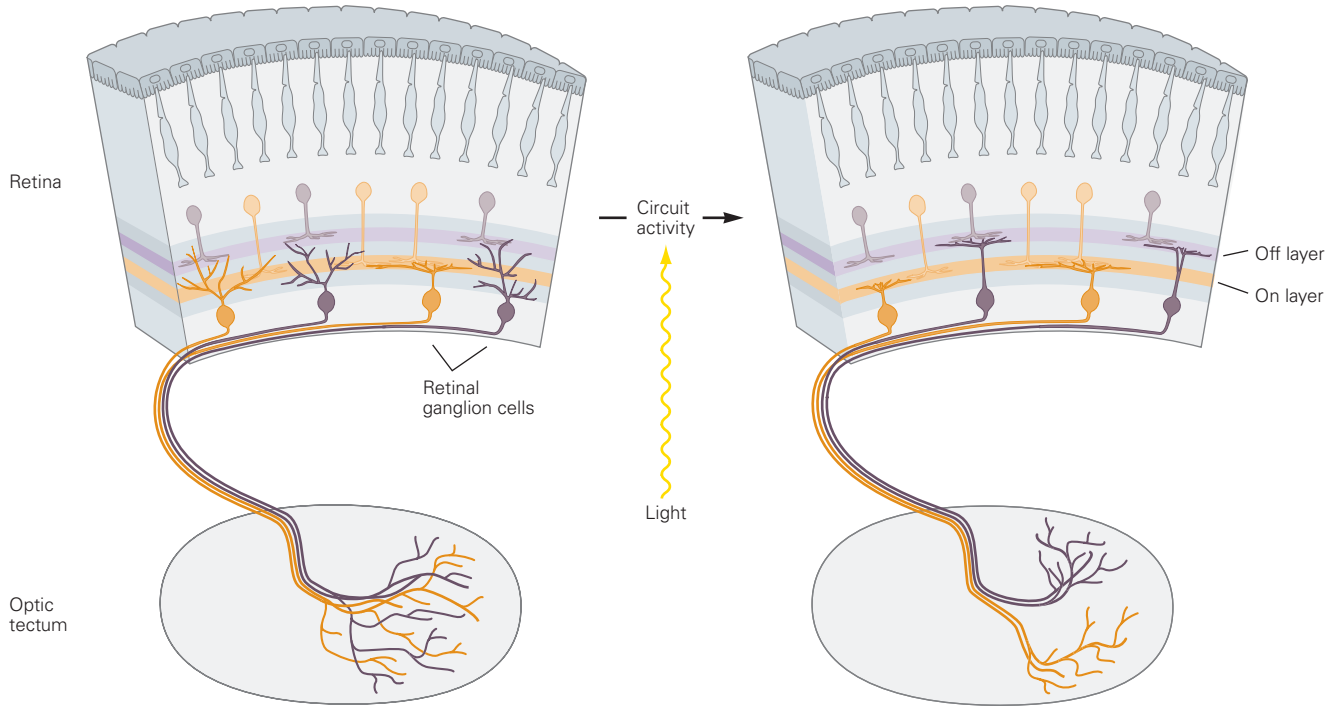

Figure 48–6 Electrical activity refines the specificity of synaptic connections of retinal ganglion cells. Some retinal ganglion cells initially form dendritic arbors that are limited to specific sublaminae in the inner plexiform layer of the retina, whereas others initially form diffuse arbors that are later pruned to form large specific patterns. Similarly, the axonal arbors of retinal ganglion cells initially innervate a large region of their target fields in the superior colliculus. This expansive axonal arbor is then refined so as to concentrate many branches in a small region. Abolishing electrical activity in retinal ganglion cells decreases the remodeling of dendritic and axonal arbors.

through neural activity. In the visual system, sharpening involves loss of synapses. We will return to this process of synapse elimination at the end of this chapter and consider its consequences for behavior in the next chapter.

In a few cases, neural activity promotes specificity in a different way, by turning an inappropriate target into an appropriate one. This mechanism has been most clearly demonstrated in skeletal muscle, where mammalian muscle fibers can be divided into several categories according to their contractile characteristics (Chapter 31). Muscle fibers of particular types express genes for distinctive isoforms of the main contractile proteins, such as myosins and troponins.

Few muscles are composed exclusively of a single type of fiber; most have fibers of all types. Yet the branches of an individual motor axon innervate muscle fibers of a single type, even in "mixed" muscles in which fibers of different types are intermingled (Figure 48–7A). This pattern implies a remarkable degree of synaptic specificity. However, matching does not always come about through recognition in the motor axon of the appropriate type of muscle fiber. The motor axon can also convert the target muscle fiber to an appropriate type. When a muscle is denervated at birth, before the properties of its fibers are fixed, a nerve that normally innervates a slow muscle can be redirected to innervate a muscle destined to become fast, and vice versa. Under these conditions, the contractile properties of the muscle are partially transformed in a direction imposed by the firing properties of the motor nerve (Figure 48–7B,C).

Different patterns of neural activity in fast and slow motor neurons are responsible for the switch in muscle properties. Most strikingly, direct electrical stimulation of a muscle with patterns normally evoked by slow or fast nerves leads to changes that are nearly as dramatic as those produced by cross-innervation (Figure 48–7D). Although activity-based conversion of the type observed at the neuromuscular junction is unlikely to be a major contributor to synaptic specificity in the central nervous system, it is likely that central axons modify the properties of their synaptic targets, contributing to the diversification of neuronal subtypes and refining connectivity imposed by recognition molecules.