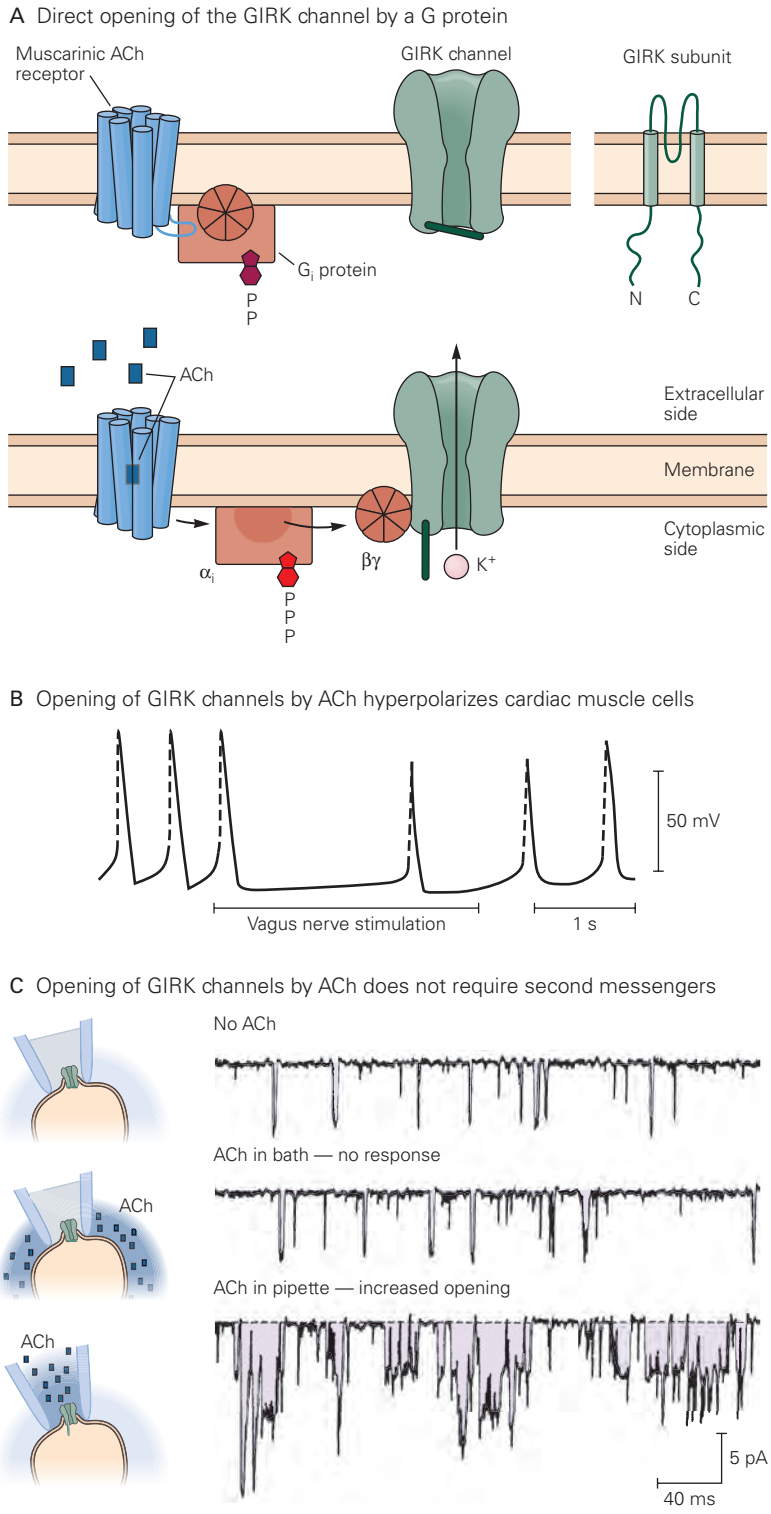

Figure 14–9 Some G proteins can open ion channels directly without employing second messengers.

A. An inward-rectifying K^+ channel (**GIRK**) is opened directly by a G protein. Binding of ACh to a muscarinic receptor causes the G_i protein and $\alpha_i\beta\gamma$ complex to dissociate; the free $\beta\gamma$-subunits bind to a cytoplasmic domain of the channel, causing the channel to open.

B. Stimulation of the parasympathetic vagus nerve releases ACh, which acts at muscarinic receptors to open GIRK channels in cardiac muscle cell membranes. The current through the GIRK channel hyperpolarizes the cells, thus slowing the heart rate. (Adapted from Toda and West 1967.)

C. Three single-channel records show that opening of GIRK channels does not involve a freely diffusible second messenger. In this experiment, the pipette contained a high concentration of K^+, which makes E_K less negative. As a result, when GIRK channels open, they generate brief pulses of inward (downward) current. In the absence of ACh, channels open briefly and infrequently (**top record**). Application of ACh in the bath (outside the pipette) does not increase channel opening in the patch of membrane under the pipette (**middle record**). This is because the free $\beta\gamma$-subunits, released by the binding of ACh to its receptor, remain tethered to the membrane near the receptor and can only activate nearby channels. The subunits are not free to diffuse to the channels under the patch pipette. The ACh must be in the pipette to activate the channel (**bottom record**). (Reproduced, with permission, from Soejima and Noma 1984. Copyright © 1984 Springer Nature.)

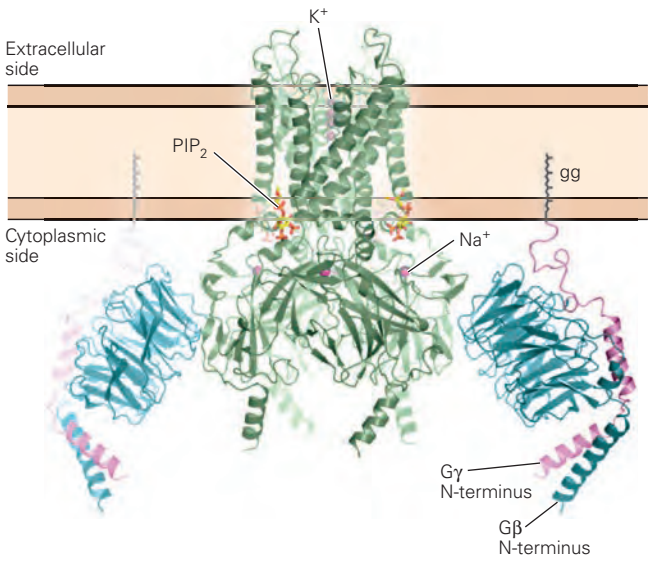

Figure 14–10 G protein βγ-subunits can directly bind and activate GIRK channels. A high-resolution structure of a GIRK channel (**green**) interacting with the G protein β-subunit (**Gβ**, **cyan**) and γ-subunit (**Gγ**, **purple**). A geranylgeranyl lipid molecule (**gg**) is attached to the C-terminus of Gγ. The structure illustrates that Na^+ ions and the phospholipid PIP_2 also bind to the channel, thereby enhancing channel opening. The **pink spheres** inside the channel represent K^+ ions. (Adapted with permission from Whorton and MacKinnon 2013. Copyright © 2013 Springer Nature.)

of voltage-gated Ca^{2+} channels in presynaptic terminals can suppress the release of neurotransmitter.

Cyclic AMP–Dependent Protein Phosphorylation Can Close Potassium Channels

In the marine mollusk *Aplysia*, a group of mechanoreceptor sensory neurons initiates defensive withdrawal reflexes in response to tactile stimuli through fast excitatory synapses with motor neurons. Certain interneurons form serotonergic synapses with these sensory neurons, and the serotonin released by the interneurons sensitizes the withdrawal reflex, enhancing the animal's response to a stimulus and thus producing a simple form of learning (Chapter 53).

The modulatory action of serotonin depends on its binding to a G protein–coupled receptor that activates a G_s protein, which elevates cAMP and thus activates PKA. This leads to the direct phosphorylation and subsequent closure of the serotonin-sensitive (or S-type) K^+ channel that acts as a resting channel (Figure 14–11). Like the closing of the M-type K^+ channel by ACh, closure of the S-type K^+ channel decreases K^+ efflux from the cell, thereby depolarizing the cell and decreasing its resting membrane conductance. Conversely, the opening of the same S-type K^+ channels can be enhanced by the neuropeptide FMRFamide, acting through 12-lipoxygenase metabolites of arachidonic acid. This enhanced channel opening leads to a slow hyperpolarizing inhibitory postsynaptic potential (IPSP) associated with an increase in resting membrane conductance.

Thus, a single channel can be regulated by distinct second-messenger pathways that produce opposite effects on neuronal excitability. Likewise, a resting K^+ channel with two pore-forming domains in each subunit (the TREK-1 channel) in mammalian neurons is dually regulated by PKA and arachidonic acid in a manner very similar to the dual regulation of the S-type channel in *Aplysia*.

Second Messengers Can Endow Synaptic Transmission with Long-Lasting Consequences

So far, we have described how synaptic second messengers alter the biochemistry of neurons for periods lasting seconds to minutes. Second messengers can also produce long-term changes lasting days to weeks as a result of alterations in a cell's expression of specific genes (Figure 14–12). Such changes in gene expression result from the ability of second-messenger cascades to control the activity of transcription factors, regulatory proteins that control mRNA synthesis.

Some transcription factors can be directly regulated by phosphorylation. For example, the cAMP response element-binding protein (CREB) is activated when phosphorylated by PKA, calcium/calmodulin-dependent protein kinases, PKC, or MAP kinases. Once activated, CREB enhances transcription by binding to specific DNA sequences, the cAMP response elements or CRE, and recruiting a component of the transcription machinery, the CREB-binding protein (CBP). CBP activates transcription by recruiting RNA polymerase II and by functioning as a histone acetylase, adding acetyl groups to certain histone lysine residues. The acetylation weakens the binding between histones and DNA, thus opening up the chromatin structure and enabling specific genes to be transcribed. The changes in transcription and chromatin structure are important for regulating neuronal development, as well as for long-term learning and memory (Chapters 53 and 54).

Modulators Can Influence Circuit Function by Altering Intrinsic Excitability or Synaptic Strength

Most of this chapter has been devoted to understanding the cellular mechanisms and signal transduction pathways that allow neuromodulator-activated

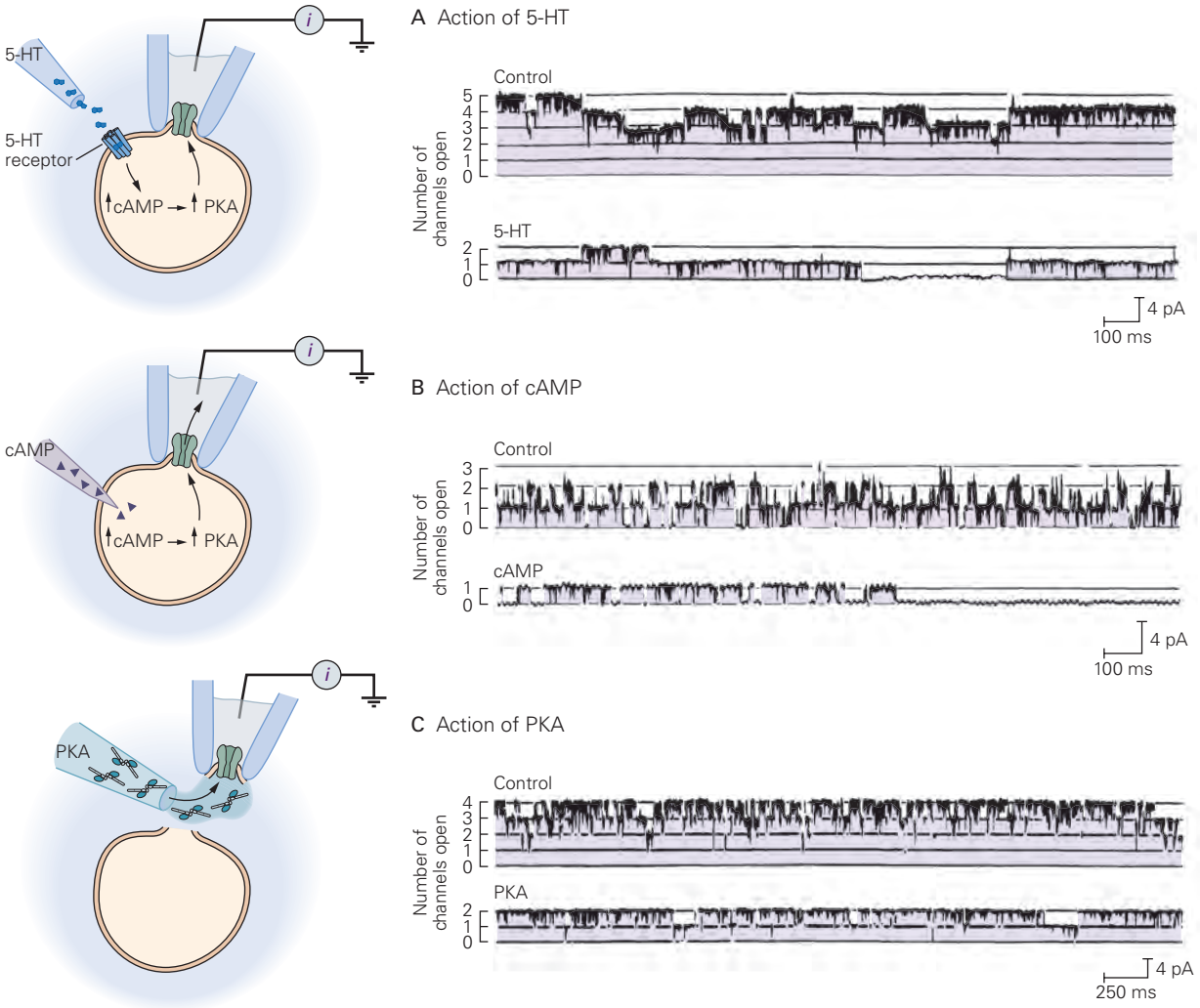

Figure 14–11 Serotonergic interneurons close a K^+ channel through the diffusible second-messenger cAMP. Serotonin (**5-HT**) produces a slow EPSP in *Aplysia* sensory neurons by closing the serotonin-sensitive or S-type K^+ channels. The 5-HT receptor is coupled to G_s, which stimulates adenylyl cyclase. The increase in cAMP activates cAMP-dependent protein kinase A (**PKA**), which phosphorylates the S-type channel, leading to its closure. Single-channel recordings illustrate the actions of 5-HT, cAMP, and PKA on the S-type channels.

A. Addition of 5-HT to the bath closes three of five S-type K^+ channels active in this cell-attached patch of membrane. The experiment implicates a diffusible messenger, as the 5-HT applied in the bath has no direct access to the S-type channels in the membrane under the pipette. Each channel opening contributes an outward (positive) current pulse. (Adapted, with permission, from Siegelbaum, Camardo, and Kandel 1982.)

B. Injection of cAMP into a sensory neuron through a microelectrode closes all three active S-type channels in this patch. The bottom trace shows the closure of the final active channel in the presence of cAMP. (Adapted, with permission, from Siegelbaum, Camardo, and Kandel 1982.)

C. Application of the purified catalytic subunit of PKA to the cytoplasmic surface of the membrane closes two out of four active S-type K^+ channels in this cell-free patch. ATP was added to the solution bathing the inside surface of the membrane to provide the source of phosphate for protein phosphorylation. (Adapted, with permission, from Shuster et al. 1985.)

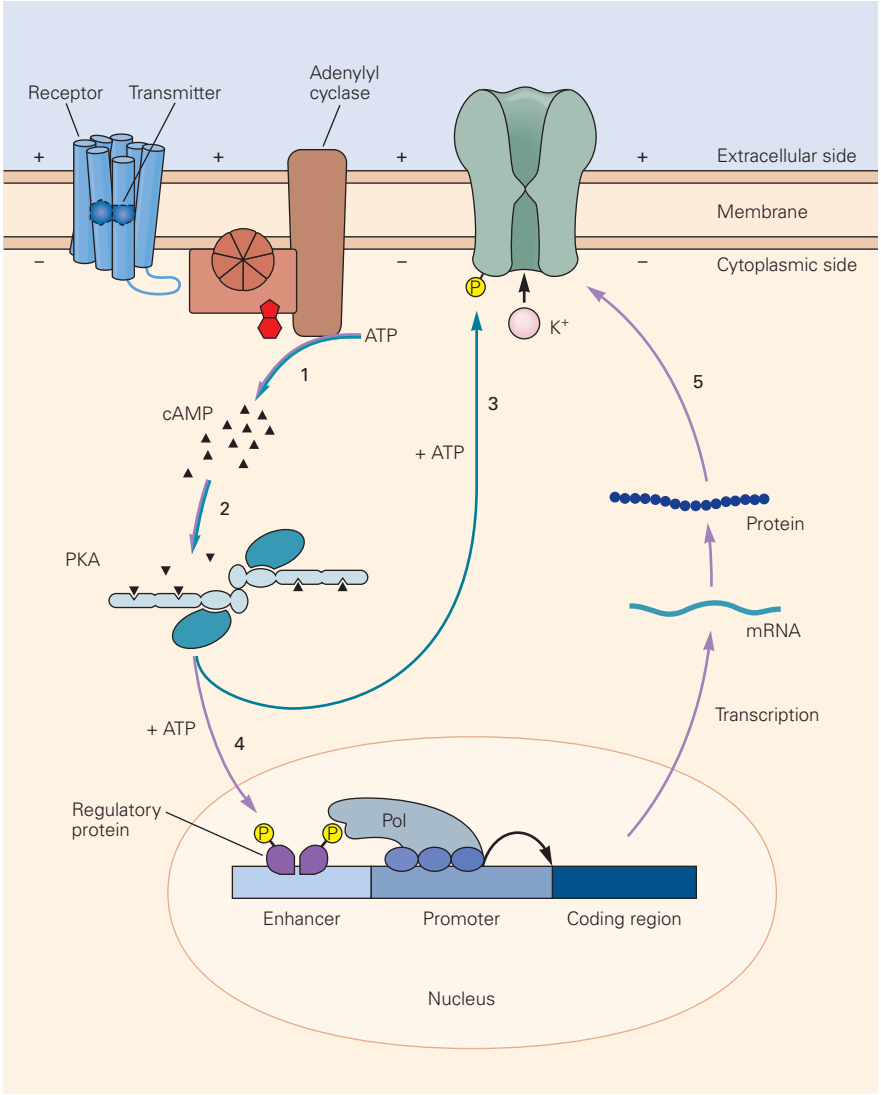

Figure 14–12 A single neurotransmitter can have either short-term or long-term effects on an ion channel. In this example, a short exposure to transmitter activates the cAMP second-messenger system (**1**), which in turn activates PKA (**2**). The kinase phosphorylates a K^+ channel; this leads to a synaptic potential that lasts for several minutes and modifies the excitability of the neuron (**3**). With sustained activation of the receptor, the kinase translocates to the nucleus, where it phosphorylates one or more transcription factors that turn on gene expression (**4**). As a result of the new protein synthesis, the synaptic actions are prolonged—closure of the channel and changes in neuronal excitability last days or longer (**5**). (**Pol**, polymerase.)

pathways to alter the activity of ion channels, receptors, and synapses in individual neurons. However, in the intact brain, modulatory transmitters released either from diffuse projections over large areas of the brain (Chapter 16) or from more locally targeted connections can alter the dynamics of brain circuits in a number of important ways. In this section, we examine one well-studied example of modulatory control of circuit function—the control of crustacean feeding behavior by the neurons of the stomatogastric ganglion to illustrate the following general properties.

1. Modulatory projection neurons or neurohormones can coordinately influence the properties of large numbers of neurons to change the state of a neural circuit or of the entire animal. For example, modulators released from a relatively small number of neurons are important in the control of the transitions between sleep and wakefulness (Chapter 44).
2. Neuromodulators act over intermediate time scales, ranging from many milliseconds to hours. Fast synaptic transmission and rapid action potential propagation are well suited for rapid computation of all kinds of processes important for behavior. Nevertheless, modulators that act over longer time scales can bias a circuit's dynamics to expand its dynamic range or to adapt it to the behavioral needs of the animal. For example, many sensory processes will evoke very different responses depending on the behavioral state of the animal, and modulators that alter synaptic strength and intrinsic excitability are often involved in such actions.

Multiple Neuromodulators Can Converge Onto the Same Neuron and Ion Channels

We have seen in our discussion of the *Aplysia* S-channel how the same ion channel can be regulated by different modulatory agents. This is a common theme, as the M-type K^+ channel is modulated by acetylcholine, substance P, and a variety of other peptides.

One particularly striking example of convergence is seen in the modulatory control of the neurons of the crustacean stomatogastric ganglion. There, a large number of structurally diverse neuropeptides converge to modulate a voltage-dependent inward current (I_{MI}). Although I_{MI} is a small current, it plays an important role in regulating excitability and the generation of plateau and burst potentials. Many neurons express a large number of different receptor types, giving these cells the ability to respond flexibly to different modulatory inputs during different brain states.

The crustacean stomatogastric ganglion (STG) contains 26 to 30 neurons and generates two rhythmic motor patterns important for feeding—the gastric rhythm and the pyloric rhythm. One set of STG neurons generates the pyloric rhythm, which is important for filtering food and is continuously active throughout the animal's life. Another set of neurons generates the gastric mill rhythm, which moves three teeth inside of the stomach that are used to chew and grind food. The gastric mill rhythm is activated in response to food and is therefore only intermittently active in vivo. Whether a particular rhythm is active at any time is under the control of a variety of neuromodulators, some of which activate the pyloric and gastric mill rhythms, while others inhibit them. These modulators can be released at specific synaptic contacts or can act diffusely as neurohormones. Interestingly, modulators can also cause individual neurons to switch between these two circuits, thereby increasing the computational power that this small number of neurons can achieve.

The fundamental circuit (the kernel) that serves as the pacemaker of the STG pyloric rhythm consists of a single anterior burster (AB) neuron and two pyloric dilator (PD) neurons. Both types of neurons make inhibitory synaptic connections with a third type of neuron, the pyloric (PY) neuron. During bursting, a slowly depolarizing pacemaker potential (slow wave) triggers a burst of action potentials in both AB and PD neurons. As these neurons are strongly coupled by electrical (gap-junction) synapses, they depolarize and synchronously fire bursts of action potentials, resulting in transient inhibition of the downstream PY neuron (Figure 14–13A).

Dopamine, which functions both as a fast neurotransmitter and as a neurohormone in crustaceans, influences feeding behavior by acting on many neurons and synapses to influence synaptic strength and neuronal and muscle excitability. For example, application of dopamine decreases the slow-wave amplitude in the PD neurons but increases the amplitude of the slow wave in the AB neurons. Ron Harris-Warrick found that dopamine modulates different sets of membrane currents in the two neurons, providing a clear example of how a single modulatory transmitter can exert distinct actions in different postsynaptic cells (Figure 14–13B).

Dopamine also alters the relative timing of the activity of these neurons. Although the PY neuron receives inhibitory input from both the AB and PD neurons, the inhibitory synaptic action from the AB neuron is faster than that from the PD neuron. Thus, dopamine, by inhibiting the PD neuron and suppressing the slow component of the IPSP, acts to speed the time course of the combined IPSP in the PY neurons (Figure 14–13A), contributing to a change in the timing of the activity of the PY neurons relative to that of the pacemaker group. Dopamine also enhances firing in the PY neuron by modulating its intrinsic *excitability*, by decreasing the transient A-type K^+ current ($I_{K,A}$) while increasing the *excitatory* slow inward current carried by the HCN channels (I_h) (Figure 14–13B). Thus, the effects of a modulator on the circuit result from its selective actions on a number of voltage-gated channels and synapses in distributed circuit elements.

Why So Many Modulators?

We now know that the STG is the direct target of 50 or more different neuromodulatory substances, including biogenic amines, amino acids, NO, and a host of neuropeptides that are released from descending modulatory projection neurons and sensory neurons and that circulate as hormones in the hemolymph. Many of these modulators are released as cotransmitters from the terminals of certain descending fibers that are activated by sensory neurons. Many neuromodulators are both released synaptically in the STG neuropil and also function as neurohormones.

Why should a small ganglion composed of only 26 to 30 neurons be modulated by so many substances? At first, it was thought that the richness of the modulatory innervation was important for producing different behaviorally relevant motor outputs. This remains true, but it is now also evident that some modulators may be used exclusively in special circumstances, such as molting, and that different modulators with similar effects ensure that important functions are preserved even if one modulatory system is lost. Thus, diverse modulators may be used in the service of both plasticity and stability.

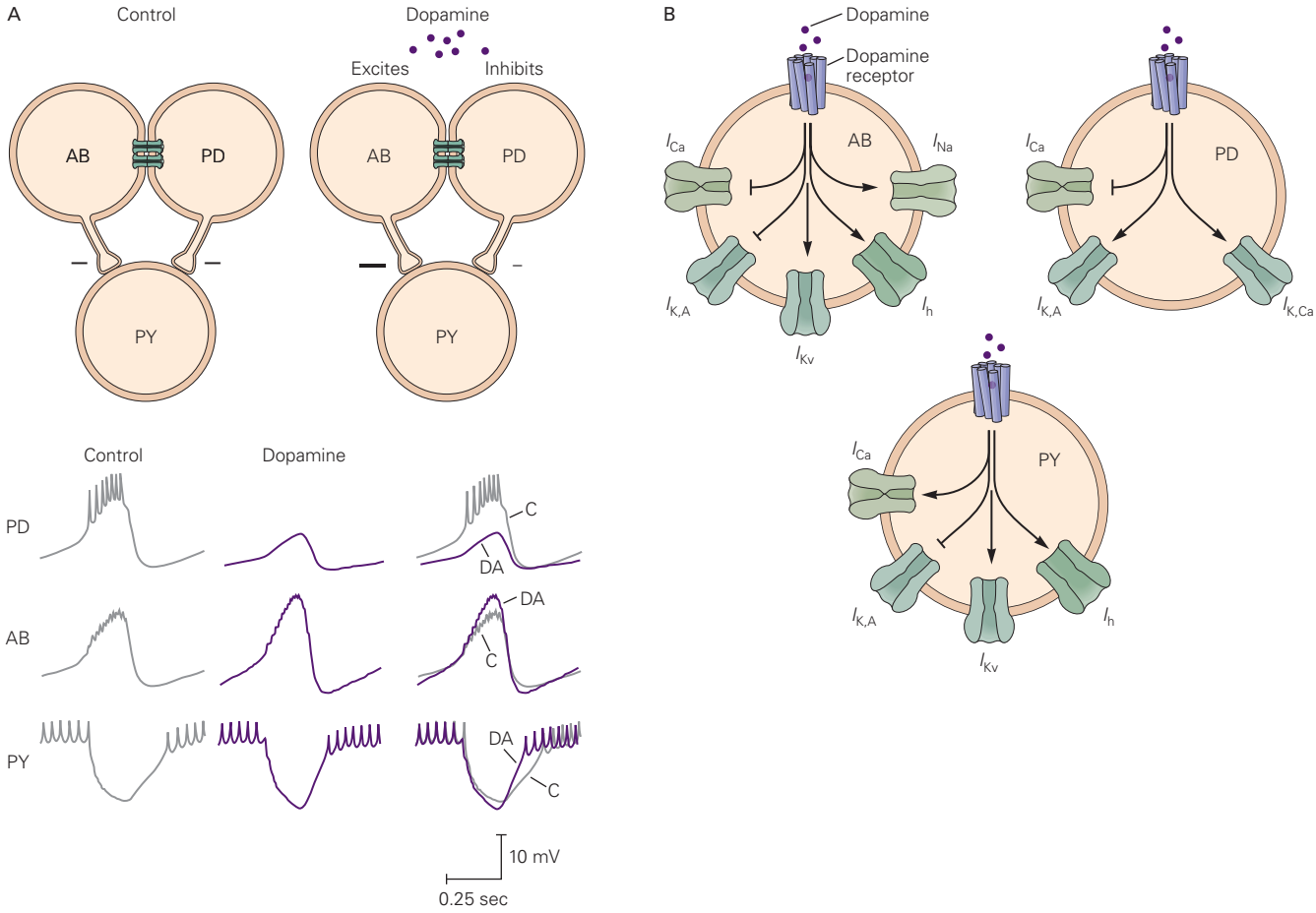

Figure 14–13 The modulatory action of dopamine on the pyloric rhythm of the lobster stomatogastric ganglion results from numerous actions.

A. A circuit diagram shows the interactions between three of the pyloric circuit neurons. The anterior burster (**AB**) and pyloric dilator (**PD**) neurons are strongly electrically coupled by gap-junction channels. Both the AB and PD neurons form inhibitory synapses with the pyloric (**PY**) neuron that generate inhibitory postsynaptic potentials (IPSPs) in this cell. Intracellular voltage recordings illustrate phases of pyloric rhythm from PD, AB, and PY neurons without dopaminergic input (control) and with dopamine. On the *right*, the voltage traces from control cells (**C**) and cells with dopaminergic input (**DA**) are overlaid. Dopamine enhances the amplitude of the slow-wave burst in the AB neuron (in this neuron, axonal action potentials are highly attenuated by the cable properties of the neuron and appear in the soma as faint ripples) but hyperpolarizes and decreases the amplitude of the slow wave in the PD neurons. These combined actions result in a shorter IPSP in the PY neuron, enabling it to fire earlier relative to the PD neurons. (Adapted, with permission, from Eisen and Marder 1984.)

B. Dopamine modulates a number of different voltage-dependent channels in the AB, PD, and PY neurons. These include Ca^{2+} currents (I_{Ca}), a calcium-activated K^+ current ($I_{K,Ca}$), an inactivating K^+ current ($I_{K,A}$), a delayed rectifier K^+ current (I_{Kv}), the hyperpolarization-activated cation current (I_h), and a persistent Na^+ current (I_{Na}). **Lines with arrowheads** indicate current increase, **lines ending in short line segment** indicate current decrease. (Adapted, with permission, from Marder and Bucher 2007. For effects of dopamine on the complete pyloric circuit, see Harris-Warrick, 2011.)

Highlights

1. Neuromodulators are substances that bind to receptors, most of which are metabotropic, to alter the excitability of neurons, the likelihood of transmitter release, or the functional state of receptors on postsynaptic neurons.
2. When neuromodulators activate second-messenger pathways, the modulator can influence the properties of ion channels and other targets at some distance from the site of release.
3. Some neuromodulatory systems have widespread and pronounced actions over many neurons and many brain areas.
4. There are two major families of metabotropic receptors: G protein–coupled receptors and receptor tyrosine kinases. Many important brain signaling molecules, such as norepinephrine,

ACh, GABA, glutamate, serotonin, dopamine, and many diverse neuropeptides, activate metabotropic receptors; many of these same substances also activate ionotropic receptors.

5. The cyclic AMP pathway is among the best-understood second-messenger signaling cascades. Metabotropic receptor activation triggers a sequence of biochemical reactions that result in activation of adenylyl cyclase, which synthesizes cAMP, which in turn activates protein kinase A. The kinase then phosphorylates target proteins, altering their functional state. Important targets for PKA include voltage- and ligand-gated ion channels as well as proteins important in vesicle release.
6. Hydrolysis of phospholipids by phospholipase C produces DAG and IP_3, which plays an important role in intracellular Ca^{2+} handling. Endocannabinoids are synthesized from lipid precursors and can act across synapses as retrograde messengers. Another generalized signaling molecule is the gas nitric oxide, which diffuses across membranes and stimulates cyclic GMP synthesis.
7. The receptor tyrosine kinases also gate ion channels indirectly in response to binding a variety of peptide hormones.
8. Neuromodulators can close ion channels, thus producing decreases in membrane conductance. The M-type current is a slowly activating voltage-gated K^+ current that underlies action potential adaptation. ACh and several neuropeptides decrease M-type current amplitude, thereby producing a slow depolarization and decreasing adaptation. The S-type K^+ channel contributes to the resting K^+ conductance of certain neurons, including a class of sensory neurons mediating the *Aplysia* gill withdrawal reflex. Closure of the channel by serotonin, acting through a cAMP signaling cascade, depolarizes the resting membrane, increases excitability, and enhances transmitter release from sensory neuron terminals. Prolonged exposure to serotonin can alter gene transcription to produce long-term changes in synaptic strength.
9. Modulators can alter the output of neuronal circuits by acting on numerous circuit targets.
10. Given that all brain neurons and synapses are likely to be modulated by one or more substances, it is remarkable that brain circuits are only rarely "overmodulated" so that they lose their function. Much additional research is needed to understand the rules that allow robust and stable network performance in the face of the modulators that allow network plasticity.
11. Except in a few notable cases such as small ganglia or the retina, it is likely that we still have only a partial catalog of the total number of neuromodulatory substances that are present and active.
12. Much of what we know about neuromodulatory actions comes from in vitro studies. Much less is known about how neuromodulatory concentrations are controlled in behaving animals.

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15

Transmitter Release

Some of the brain's most remarkable abilities, such as learning and memory, are thought to emerge from the elementary properties of chemical synapses, where the presynaptic cell releases chemical transmitters that activate receptors in the membrane of the postsynaptic cell. At most central synapses, transmitter is released from the presynaptic cell at presynaptic boutons, varicosities along the axon (like beads on a string) filled with synaptic vesicles and other organelles that contact postsynaptic targets. At other synapses, including the neuromuscular junction, transmitter is released from presynaptic terminals at the end of the axon. For convenience, we will refer to both types of release sites as presynaptic terminals. In the last three chapters, we saw how postsynaptic receptors control ion channels that generate the postsynaptic potential. Here we consider how electrical and biochemical events in the presynaptic terminal lead to the rapid release of small-molecule neurotransmitters, such as acetylcholine (ACh), glutamate, and γ-aminobutyric acid (GABA), that underlie fast synaptic transmission. In the next chapter, we examine the chemistry of the neurotransmitters themselves as well as the biogenic amines (serotonin, norepinephrine, and dopamine) and neuropeptides, which underlie slower forms of intercellular signaling.

Transmitter Release Is Regulated by Depolarization of the Presynaptic Terminal

What event at the presynaptic terminal leads to the release of transmitter? Bernard Katz and Ricardo Miledi first demonstrated the importance of depolarization of the presynaptic membrane. For this purpose, they used