1 Chemical Neurotransmission

Anatomical versus Chemical Basis of Neurotransmission 1
General Structure of a Neuron 2
Principles of Chemical Neurotransmission 5
Neurotransmitters 5
Neurotransmission: Classic, Retrograde, and Volume 6
Excitation–Secretion Coupling 8
Signal Transduction Cascades 9
Overview 9
Forming a Second Messenger 11
Beyond the Second Messenger to Phosphoprotein Messengers 13
Beyond the Second Messenger to a Phosphoprotein Cascade Triggering Gene Expression 15
How Neurotransmission Triggers Gene Expression 18
Molecular Mechanism of Gene Expression 18
Epigenetics 23
What Are the Molecular Mechanisms of Epigenetics? 23
How Epigenetics Maintains or Changes the Status Quo 24
A Brief Word about RNA 26
Alternative Splicing 26
RNA Interference 26
Summary 28

Modern psychopharmacology is largely the story of chemical neurotransmission. To understand the actions of drugs on the brain, to grasp the impact of diseases upon the central nervous system, and to interpret the behavioral consequences of psychiatric medicines, one must be fluent in the language and principles of chemical neurotransmission. The importance of this fact cannot be overstated for the student of psychopharmacology. This chapter forms the foundation for the entire book, and the roadmap for one's journey through one of the most exciting topics in science today, namely the neuroscience of how disorders and drugs act upon the central nervous system.

ANATOMICAL VERSUS CHEMICAL BASIS OF NEUROTRANSMISSION

What is neurotransmission? Neurotransmission can be described in many ways: anatomically, chemically, electrically. The *anatomical* basis of neurotransmission is neurons (Figures 1-1 to 1-3) and the connections between them, called synapses (Figure 1-4), sometimes also called the *anatomically addressed nervous system*, a complex of "hard-wired" synaptic connections between neurons, not unlike millions of telephone wires within thousands upon thousands of cables. The *anatomically addressed brain* is thus a complex wiring diagram, ferrying electrical impulses to wherever the "wire" is plugged in (i.e., at a synapse). Synapses can form on many parts of a neuron, not just from the axon of one neuron to the dendrite of another neuron as axodendritic synapses, but also from the axon of one neuron to the soma of another neuron as axosomatic synapses, and even from one neuron's axon to another neuron's axon, especially at the beginning and at the end of the receiving neuron's axons (axoaxonic synapses) (Figure 1-2). Such synapses are said to be "asymmetric" since communication is structurally designed to be in one direction, i.e., anterograde from the axon of the first neuron to the dendrite, soma, or axon of the second neuron (Figures 1-2 and 1-3). This means that there are presynaptic elements that differ from postsynaptic elements (Figure 1-4). Specifically, a neurotransmitter is packaged in the presynaptic nerve terminal like ammunition in a loaded gun, and then fired at the postsynaptic neuron to target its receptors.

Neurons are the cells of chemical communication in the brain. Human brains are comprised of tens of billions of neurons, and each is linked to thousands of other neurons. Thus, the brain has trillions of specialized connections known as synapses. Neurons have many sizes, lengths, and shapes that determine their functions. Localization within the brain also determines function. When neurons malfunction, behavioral symptoms may

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

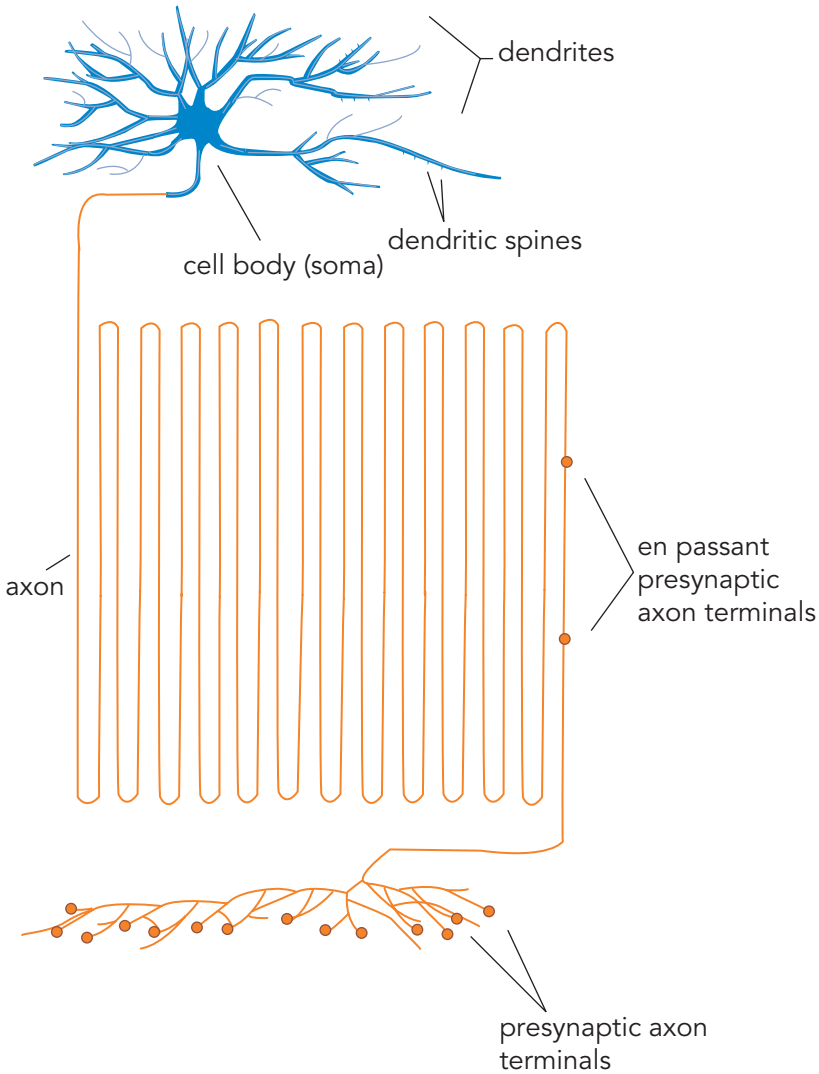


Figure 1-1 General structure of a neuron. This is an artist's conception of the generic structure of a neuron. All neurons have a cell body known as the soma, which is the command center of the nerve and contains the nucleus of the cell. All neurons are also set up structurally to both send and receive information. Neurons send information via an axon that forms presynaptic terminals as the axon passes by (en passant) or as the axon ends.

occur. When drugs alter neuronal function, behavioral symptoms may be relieved, worsened, or produced.

General Structure of a Neuron

Although this textbook will often portray neurons with a generic structure (such as that shown in Figures 1-1 to 1-3), the truth is that many neurons have unique structures depending upon where in the brain they are located and what their function is. On the one hand, all neurons have a cell body known as the soma, and are set up structurally to receive information from other neurons through dendrites, sometimes via spines on the dendrites and often through an elaborately branching "tree" of dendrites (Figure 1-2). Neurons are also set up structurally to send information to other neurons via an axon that forms presynaptic terminals as the axon passes by (en passant, Figure 1-1) or as the axon ends (presynaptic axon terminals, Figures 1-1 through 1-4).

Neurotransmission has an *anatomical* infrastructure, but it is fundamentally a very elegant *chemical* operation. Complementary to the anatomically addressed nervous system is thus the *chemically addressed nervous system*, which forms the chemical basis of neurotransmission: namely, how chemical signals are coded, decoded, transduced, and sent along the way. Understanding the principles of chemical

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

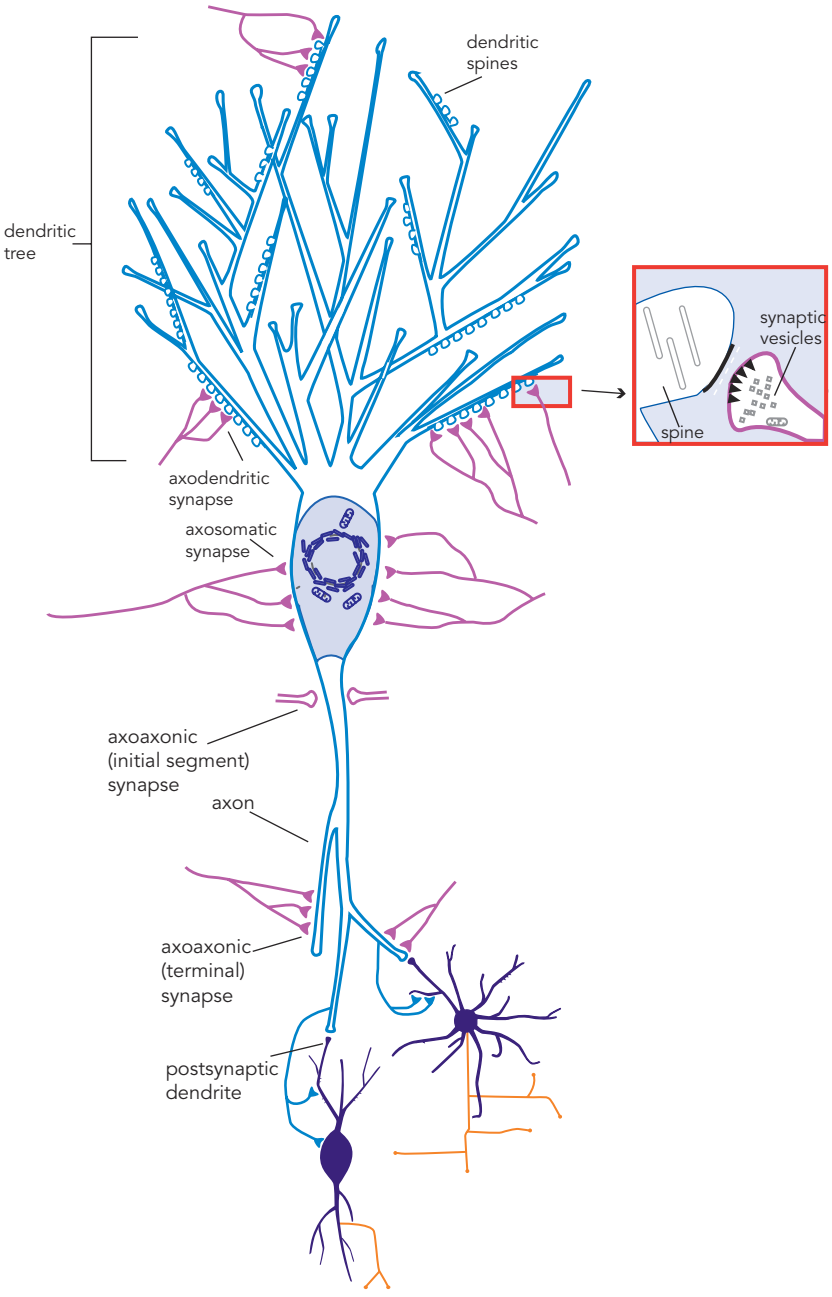


Figure 1-2 Axodendritic, axosomatic, and axoaxonic connections. After neurons migrate, they form synapses. As shown in this figure, synaptic connections can form not just between the axon and dendrites of two neurons (axodendritic) but also between the axon and the soma (axosomatic) or the axons of the two neurons (axoaxonic). Communication is anterograde from the axon of the first neuron to the dendrite, soma, or axon of the second neuron.

neurotransmission is a fundamental requirement for grasping how psychopharmacological agents work, because these agents target key molecules involved in neurotransmission. Drug targeting of specific chemical sites that influence neurotransmission is discussed in Chapters 2 and 3.

Understanding the chemically addressed nervous system is also a prerequisite for becoming a "neurobiologically informed" clinician: that is, being able to translate exciting new findings on brain circuitry, functional neuroimaging, and genetics into clinical practice, and potentially improving the manner in which

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

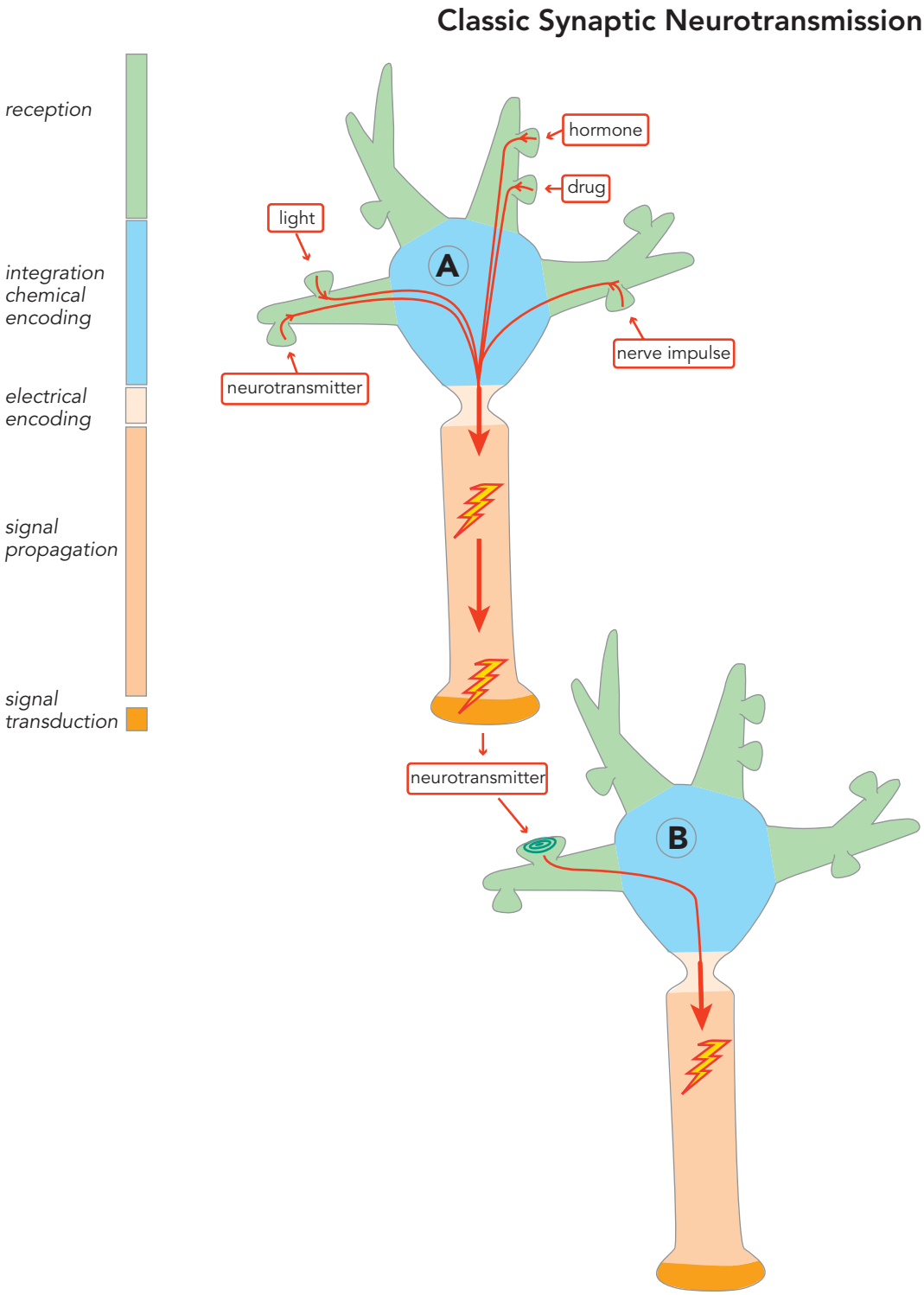


Figure 1-3 **Classic synaptic neurotransmission.** In classic synaptic neurotransmission, stimulation of a presynaptic neuron (e.g., by neurotransmitters, light, drugs, hormones, nerve impulses) causes electrical impulses to be sent to its axon terminal. These electrical impulses are then converted into chemical messengers and released to stimulate the receptors of a postsynaptic neuron. Thus, although communication *within* a neuron can be electrical, communication *between* neurons is chemical.

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

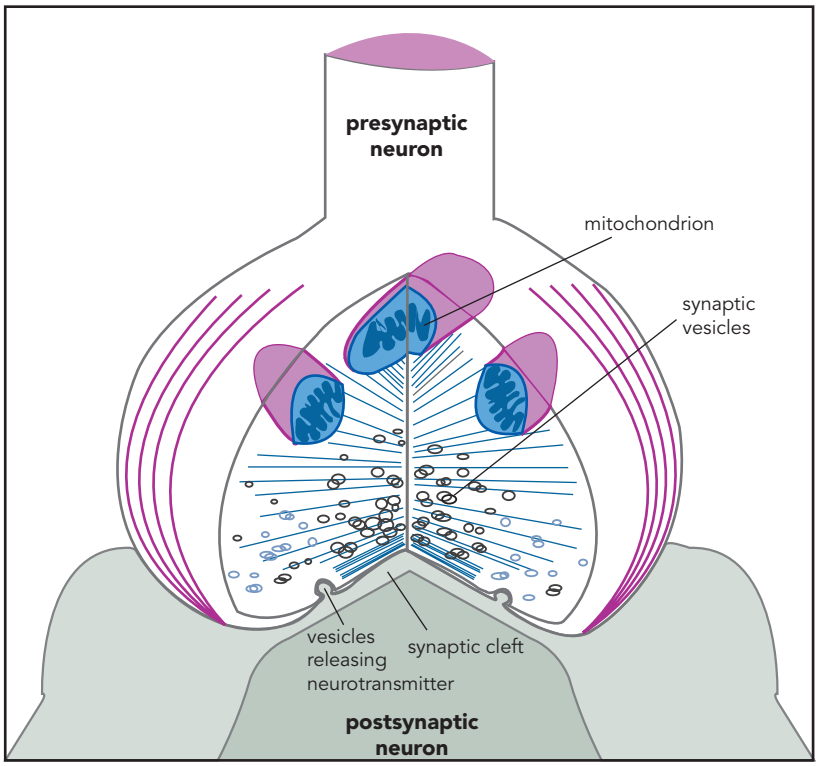


Figure 1-4 Enlarged synapse. The synapse is enlarged conceptually here showing the specialized structures that enable chemical neurotransmission to occur. Specifically, a presynaptic neuron sends its axon terminal to form a synapse with a postsynaptic neuron. Energy for neurotransmission from the presynaptic neuron is provided by mitochondria there. Chemical neurotransmitters are stored in small vesicles, ready for release upon firing of the presynaptic neuron. The synaptic cleft is the gap between the presynaptic neuron and the postsynaptic neuron; it contains proteins and scaffolding and molecular forms of "synaptic glue" to reinforce the connection between the neurons. Receptors are present on both sides of this cleft and are key elements of chemical neurotransmission.

psychiatric disorders and their symptoms are diagnosed and treated. The chemistry of neurotransmission in specific brain regions and how these principles are applied to various specific psychiatric disorders, treated with various specific psychotropic drugs, are discussed throughout the rest of the book.

PRINCIPLES OF CHEMICAL NEUROTRANSMISSION

Neurotransmitters

There are more than a dozen known or suspected neurotransmitters in the brain. For psychopharmacologists, it is particularly important to know the six key neurotransmitter systems targeted by psychotropic drugs:

serotonin
norepinephrine
dopamine
acetylcholine
glutamate
GABA (γ-aminobutyric acid)

Each is discussed in detail in the clinical chapters related to the specific drugs that target them.

Other neurotransmitters that are also important neurotransmitters and neuromodulators, such as histamine and various neuropeptides and hormones, are mentioned in brief throughout the relevant clinical chapters in this textbook.

Some neurotransmitters are very similar to drugs and have been called "God's pharmacopeia." For example, it is well known that the brain makes its own morphine (i.e., β-endorphin) and its own marijuana (i.e., endocannabinoids). The brain may even make its own Prozac, its own Xanax, and and its own hallucinogens! Drugs often mimic the brain's natural neurotransmitters and some drugs have been discovered prior to the natural neurotransmitter. Thus, morphine was used in clinical

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

practice before the discovery of β-endorphin; marijuana was smoked before the discovery of cannabinoid receptors and endocannabinoids; the benzodiazepines Valium (diazepam) and Xanax (alprazolam) were prescribed before the discovery of benzodiazepine receptors; and the antidepressants Elavil (amitriptyline) and Prozac (fluoxetine) entered clinical practice before molecular clarification of the serotonin transporter site. This underscores the point that the great majority of drugs that act in the central nervous system act upon the process of neurotransmission. Indeed, this apparently occurs at times in a manner that can mimic the actions of the brain itself, when the brain uses its own chemicals.

Input to any neuron can involve many different neurotransmitters coming from many different neuronal circuits. Understanding these inputs to neurons within functioning circuits can provide a rational basis for selecting and combining therapeutic agents. This theme is discussed extensively in each chapter on the various psychiatric disorders. The idea is that for the modern psychopharmacologist to influence abnormal neurotransmission in patients with psychiatric disorders, it may be necessary to target neurons in specific circuits. Since these networks of neurons send and receive information via a variety of neurotransmitters, it may therefore be not only rational but necessary to use multiple drugs with multiple neurotransmitter actions for patients with psychiatric disorders, especially if single agents with single neurotransmitter mechanisms are not effective in relieving symptoms.

Neurotransmission: Classic, Retrograde, and Volume

Classic neurotransmission begins with an electrical process by which neurons send electrical impulses from one part of the cell to another part of the same cell via their axons (see neuron A of Figure 1-3). However, these electrical impulses do not jump directly to other neurons. Classic neurotransmission between neurons involves one neuron hurling a chemical messenger, or neurotransmitter, at the receptors of a second neuron (see the synapse between neuron A and neuron B in Figure 1-3). This happens frequently but not exclusively at the sites of synaptic connections. In the human brain, a hundred billion neurons each make thousands of synapses with other neurons for an estimated trillion chemically neurotransmitting synapses.

Communication *between* all these neurons at synapses is chemical, not electrical. That is, an electrical impulse in the first neuron is converted to a chemical signal at the synapse between it and a second neuron, in a process known as excitation–secretion coupling, the first stage of chemical neurotransmission. This occurs predominantly but not exclusively in one direction, from the *presynaptic* axon terminal to a second *postsynaptic* neuron (Figures 1-2 and 1-3). Finally, neurotransmission continues in the second neuron either by converting the chemical information from the first neuron back into an electrical impulse in the second neuron, or, perhaps more elegantly, by the chemical information from the first neuron triggering a cascade of further chemical messages within the second neuron to change that neuron's molecular and genetic functioning (Figure 1-3).

An interesting twist to chemical neurotransmission is the discovery that postsynaptic neurons can also "talk back" to their presynaptic neurons. They can do this via retrograde neurotransmission from the second neuron to the first at the synapse between them (Figure 1-5, right panel). Chemicals produced specifically as retrograde neurotransmitters at some synapses include the endocannabinoids (EC, also known as "endogenous marijuana"), which are synthesized in the postsynaptic neuron. They are then released and diffuse to presynaptic cannabinoid receptors such as the CB1 or cannabinoid 1 receptor (Figure 1-5, right panel). Another retrograde neurotransmitter is the gaseous neurotransmitter nitric oxide (NO), which is synthesized postsynaptically and then diffuses out of the postsynaptic membrane and into the presynaptic membrane to interact with cyclic guanosine monophosphate (cGMP)-sensitive targets there (Figure 1-5, right panel). A third type of retrograde neurotransmitter are neurotrophic factors such as nerve growth factor (NGF), which is released from postsynaptic sites, and then diffuses to the presynaptic neuron, where it is taken up into vesicles, and transported all the way back to the cell nucleus via retrograde transport systems to interact with the genome there (Figure 1-5, right panel). What these retrograde neurotransmitters have to say to the presynaptic neuron and how this modifies or regulates the communication between pre and postsynaptic neuron are subjects of intense active investigation.

In addition to "reverse" or retrograde neurotransmission at synapses, some neurotransmission does not need a synapse at all! Neurotransmission without a synapse is called *volume neurotransmission*, or nonsynaptic diffusion neurotransmission (examples are shown in Figures 1-6 through 1-8). Chemical messengers

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

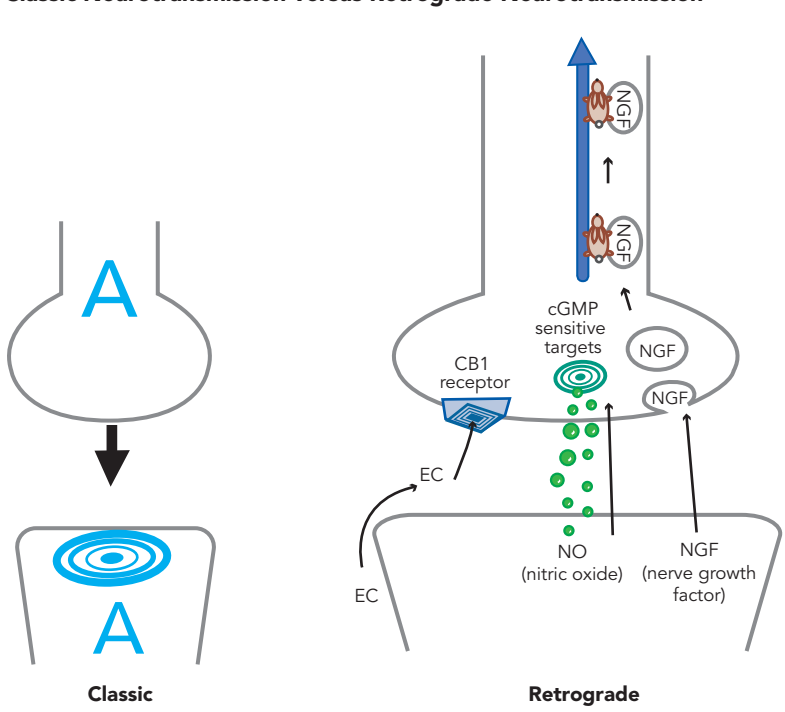


Figure 1-5 Retrograde neurotransmission. Not all neurotransmission is classic or anterograde or from top to bottom – namely, presynaptic to postsynaptic (left). Postsynaptic neurons may also communicate with presynaptic neurons from the bottom to the top via retrograde neurotransmission, from postsynaptic neuron to presynaptic neuron (right). Some neurotransmitters produced specifically as retrograde neurotransmitters at some synapses include the endocannabinoids (ECs, or endogenous marijuana), which are synthesized in the postsynaptic neuron, released, and diffuse to presynaptic cannabinoid receptors such as the cannabinoid 1 receptor (CB1); the gaseous neurotransmitter nitric oxide (NO), which is synthesized postsynaptically and then diffuses both out of the postsynaptic membrane and into the presynaptic membrane to interact with cyclic guanosine monophosphate (cGMP)-sensitive targets there; and neurotrophic factors such as nerve growth factor (NGF), which is released from postsynaptic sites and diffuses to the presynaptic neuron, where it is taken up into vesicles and transported all the way back to the cell nucleus via retrograde transport systems to interact with the genome there.

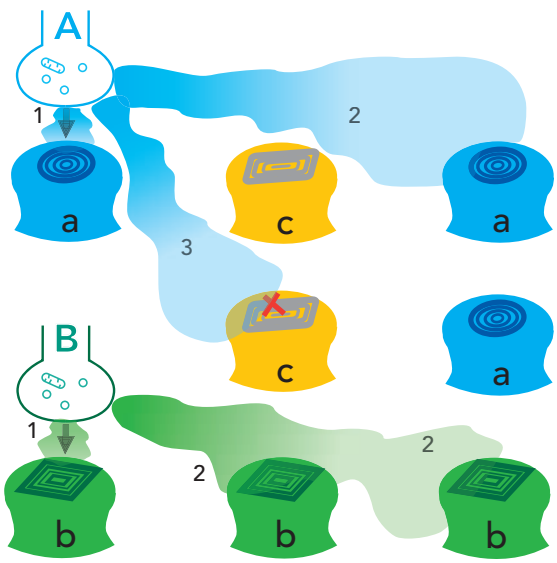


Figure 1-6 Volume neurotransmission. Neurotransmission can also occur without a synapse; this is called volume neurotransmission or nonsynaptic diffusion. In this figure, two anatomically addressed synapses (neurons A and B) are shown communicating with their corresponding postsynaptic receptors (a and b; 1). However, there are also receptors for neurotransmitter A, neurotransmitter B, and neurotransmitter C, which are distant from the synaptic connections of the anatomically addressed nervous system. If neurotransmitter A or B can diffuse away from its synapse before it is destroyed, it will be able to interact with other matching receptor sites distant from its own synapse (2). If neurotransmitter A or B encounters a different receptor not capable of recognizing it (receptor c), it will not interact with that receptor even if it diffuses there (3). Thus, a chemical messenger sent by one neuron to another can spill over by diffusion to sites distant from its own synapse. Neurotransmission can occur at a compatible receptor within the diffusion radius of the matched neurotransmitter. This is analogous to modern communication with cellular telephones, which function within the transmitting radius of a given cell. This concept is called the chemically addressed nervous system, in which neurotransmission occurs in chemical "puffs." The brain is thus not only a collection of wires but also a sophisticated "chemical soup."

sent by one neuron to another can spill over to sites distant to the synapse by diffusion (Figure 1-6). Thus, neurotransmission can occur at any compatible receptor within the diffusion radius of the neurotransmitter, not unlike modern communication with cellular telephones, which function within the transmitting radius of a given cell tower (Figure 1-6). This concept is part of the chemically addressed nervous system, and here neurotransmission occurs in chemical "puffs" (Figures 1–6 through 1–8). The brain is thus not only a collection of wires, but also a sophisticated "chemical soup." The chemically addressed nervous system is particularly

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

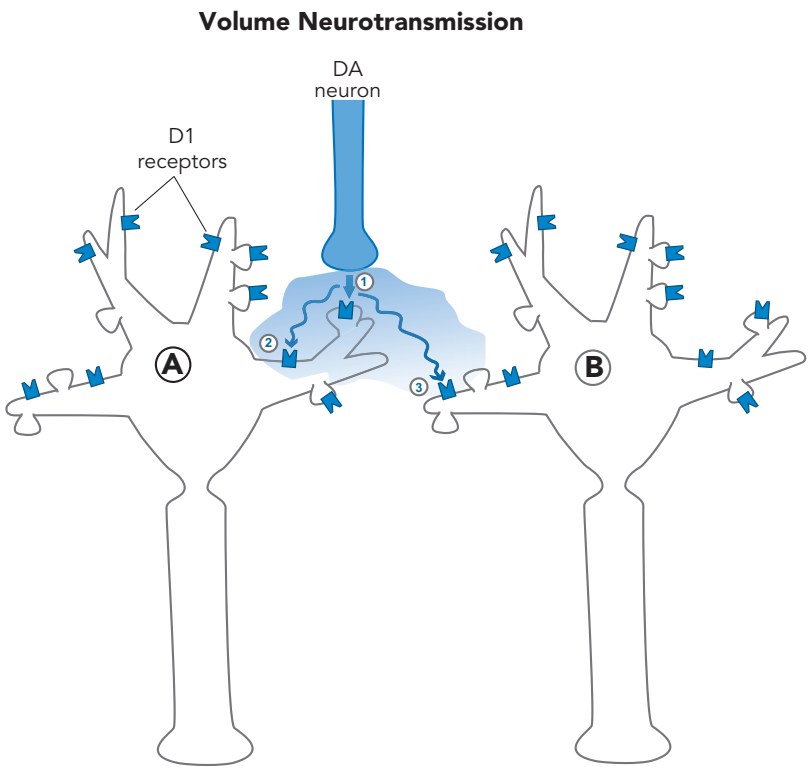


Figure 1-7 Volume neurotransmission: dopamine. An example of volume neurotransmission would be that of dopamine (DA) in the prefrontal cortex. Since there are few dopamine reuptake pumps in the prefrontal cortex, dopamine is available to diffuse to nearby receptor sites. Thus, dopamine released from a synapse (arrow 1) targeting postsynaptic neuron A is free to diffuse further in the absence of a reuptake pump and can reach dopamine receptors on that same neuron but outside of the synapse from which it was released, on neighboring dendrites (arrow 2). Shown here is dopamine also reaching extrasynaptic receptors on a neighboring neuron (arrow 3).

important in mediating the actions of drugs that act at various neurotransmitter receptors, since such drugs will act wherever there are relevant receptors, and not just where such receptors are innervated with synapses by the anatomically addressed nervous system. Modifying volume neurotransmission may indeed be a major way in which several psychotropic drugs work in the brain.

A good example of volume neurotransmission is dopamine action in the prefrontal cortex. Here there are very few dopamine reuptake transport pumps (dopamine transporters or DATs) to terminate the action of dopamine released in the prefrontal cortex during neurotransmission. This is much different from other brain areas, such as the striatum, where dopamine reuptake pumps are present in abundance. Thus, when dopamine neurotransmission occurs at a synapse in the prefrontal cortex, dopamine is free to spill over from that synapse and diffuse to neighboring dopamine receptors and stimulate them, even though there is no synapse at these "spillover" sites (Figure 1-7).

Another important example of volume neurotransmission is at the sites of autoreceptors on monoamine neurons (Figure 1-8). At the somatodendritic end of the neuron (top of the neurons in Figure 1-8) are autoreceptors that inhibit the release of neurotransmitter from the axonal end of the neuron (bottom of the neurons in Figure 1-8). Although some recurrent axon collaterals and other monoamine neurons may directly innervate somatodendritic receptors, these so-called somatodendritic autoreceptors also apparently receive neurotransmitter from dendritic release (Figure 1-8, middle and right panels). There is no synapse here, no synaptic vesicles, just neurotransmitter apparently "leaked" from the neuron's dendrites upon its own receptors in a mechanism that is still being clarified. The nature of a neuron's regulation by its somatodendritic autoreceptors is a subject of intense interest, and is theoretically linked to the mechanism of action of many antidepressants, as will be explained later in Chapter 7. The take-home point here is that not all chemical neurotransmission occurs at synapses.

Excitation–Secretion Coupling

An electrical impulse in the first – or presynaptic – neuron is converted into a chemical signal at the synapse by a process known as *excitation–secretion coupling*. Once an

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

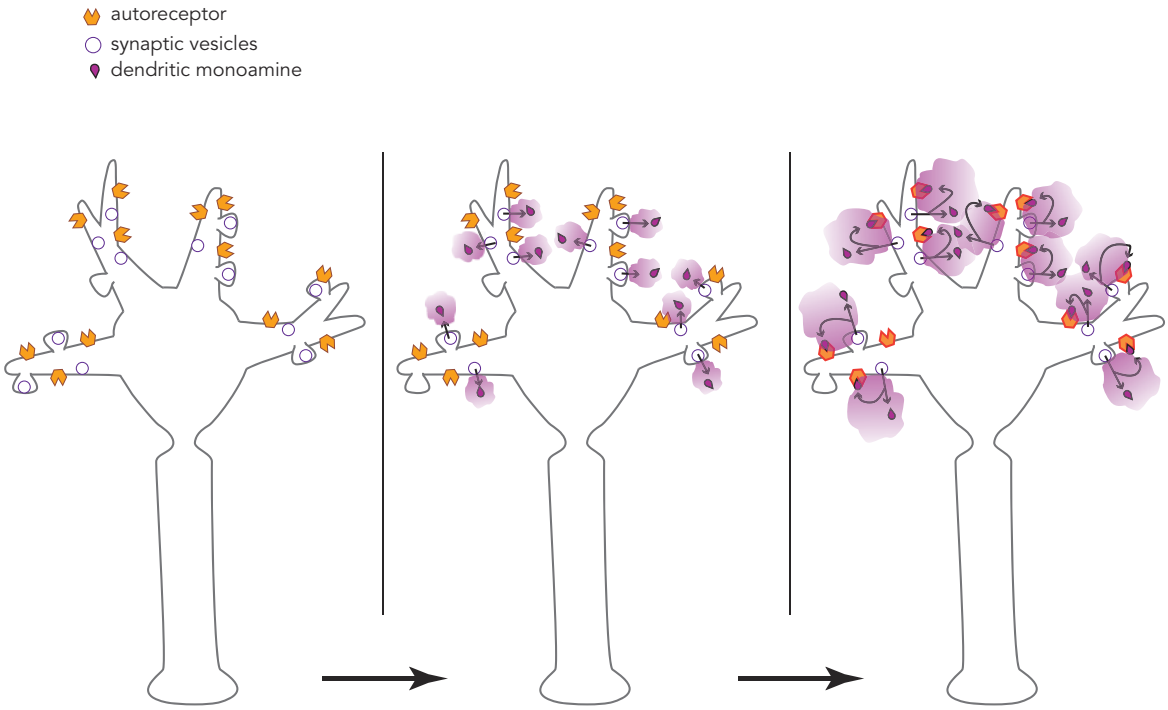


Figure 1-8 Volume neurotransmission: monoamine autoreceptors. Another example of volume neurotransmission could involve autoreceptors on monoamine neurons. Autoreceptors located on the dendrites and soma of a neuron (at the top of the neuron in the left panel) normally inhibit release of neurotransmitter from the axon of that neuron (at the bottom of the neuron in the left panel), and thus inhibit impulse flow through that neuron from top to bottom. Monoamines released from the dendrites of this neuron (at the top of the neuron in the middle panel), then bind to these autoreceptors (at the top of the neuron in the right panel) and would inhibit neuronal impulse flow in that neuron (from the bottom of the neuron in the right panel). This action occurs due to volume neurotransmission and despite the absence of synaptic neurotransmission in the somatodendritic areas of these neurons.

electrical impulse invades the presynaptic axon terminal, it causes the release of chemical neurotransmitter stored there (Figures 1-3 and 1-4). Electrical impulses open ion channels – both *voltage-sensitive sodium channels* (VSSCs) and *voltage-sensitive calcium channels* (VSCCs) – by changing the ionic charge across neuronal membranes. As sodium flows into the presynaptic nerve through sodium channels in the axon membrane, the electrical charge of the action potential moves along the axon until it reaches the presynaptic nerve terminal where it also opens calcium channels. As calcium flows into the presynaptic nerve terminal, it causes synaptic vesicles anchored to the inner membrane to spill their chemical contents into the synapse. The way is paved for chemical communication by previous synthesis of neurotransmitter and storage of neurotransmitter in the first neuron's presynaptic axon terminal.

Excitation–secretion coupling is thus the way that the neuron transduces an electrical stimulus into a chemical event. This happens very quickly once the electrical impulse enters the presynaptic neuron. It is also possible for the neuron to transduce a chemical message from a presynaptic neuron back into an electrical chemical message in the postsynaptic neuron by opening ion channels linked to neurotransmitters there. This also happens very quickly when chemical neurotransmitters open ion channels that change the flow of charge into the neuron, and ultimately, action potentials in the postsynaptic neuron. Thus, the process of neurotransmission is constantly transducing chemical signals into electrical signals, and electrical signals back into chemical signals.

SIGNAL TRANSDUCTION CASCADES

Overview

Neurotransmission can be seen as part of a much larger process than just the communication of a presynaptic axon with a postsynaptic neuron at the synapse between them. That is, neurotransmission can also be seen as

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

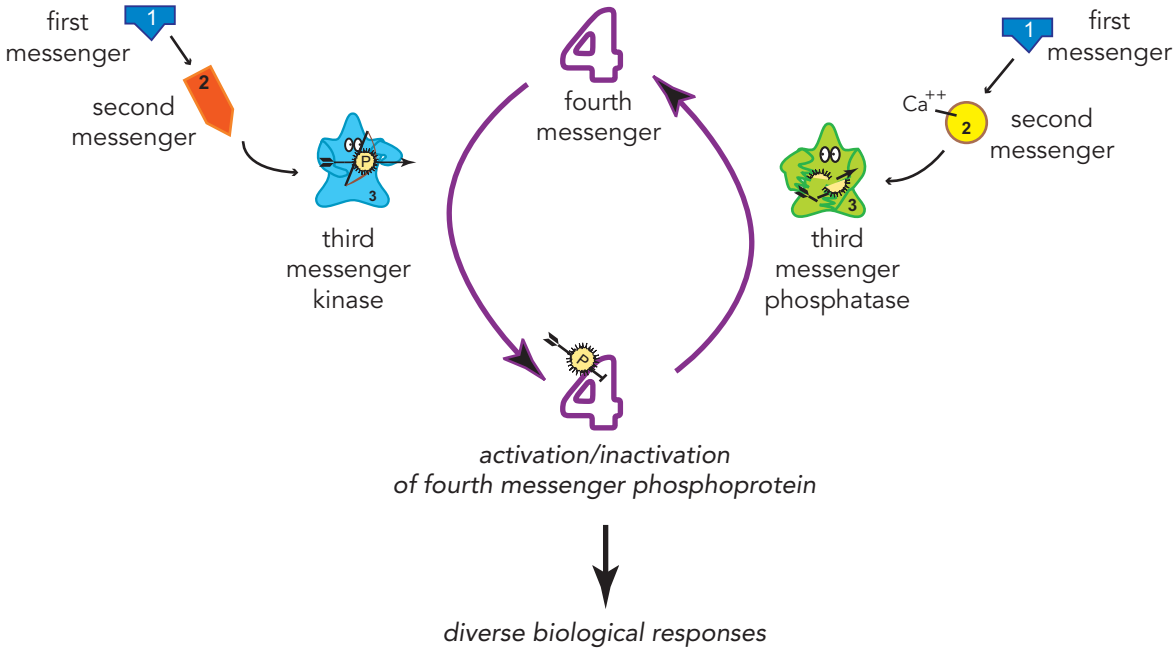


Figure 1-9 Signal transduction cascade. The cascade of events that occurs following stimulation of a postsynaptic receptor is known as signal transduction. Signal transduction cascades can activate third-messenger enzymes known as kinases, which add phosphate groups to proteins to create phosphoproteins (on the left). Other signal transduction cascades can activate third-messenger enzymes known as phosphatases, which remove phosphates from phosphoproteins (on the right). The balance between kinase and phosphatase activity, signaled by the balance between the two neurotransmitters that activate each of them, determines the degree of downstream chemical activity that gets translated into diverse biological responses, such as gene expression and synaptogenesis.

communication from the genome of the presynaptic neuron (neuron A of Figure 1-3) to the genome of the postsynaptic neuron (neuron B of Figure 1-3), and then back from the genome of the postsynaptic neuron to the genome of the presynaptic neuron via retrograde neurotransmission (right panel of Figure 1-5). Such a process involves long strings of chemical messages within both presynaptic and postsynaptic neurons, called signal transduction cascades.

Signal transduction cascades triggered by chemical neurotransmission thus involve numerous molecules, starting with neurotransmitter first messenger, and proceeding to second, third, fourth, and more messengers (Figures 1-9 through 1-30). The initial events occur in less than a second, but the long-term consequences are mediated by downstream messengers that take hours to days to activate, yet can last for many days or even for the lifetime of a synapse or neuron (Figure 1-10). Signal transduction cascades are somewhat akin to a molecular "pony express" with specialized molecules acting as a sequence of riders, handing off the message to the next specialized molecule, until the message has reached a functional destination, such as gene expression or activation of otherwise "sleeping" and inactive molecules (see for example, Figures 1-9 through 1-19).

An overview of such a molecular "pony express," from first-messenger neurotransmitter through several "molecular riders" to the production of diverse biological responses, is shown in Figure 1-9. Specifically, a first-messenger neurotransmitter on the left activates the production of a chemical second messenger that in turn activates a third messenger, namely an enzyme known as a kinase that adds phosphate groups to fourth-messenger proteins to create phosphoproteins (Figure 1-9, left). Another signal transduction cascade is shown on the right with a first-messenger neurotransmitter opening an ion channel that allows calcium to enter the neuron and act as the second messenger for this cascade system (Figure 1-9, right). Calcium then activates a different third messenger on the right, namely an enzyme known as a phosphatase that removes phosphate groups from fourth-messenger phosphoproteins and thus reverses the actions of the third messenger on the left. The balance between kinase and phosphatase activity, signaled by the balance between the two neurotransmitters that activate each of them, determines the degree of downstream

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

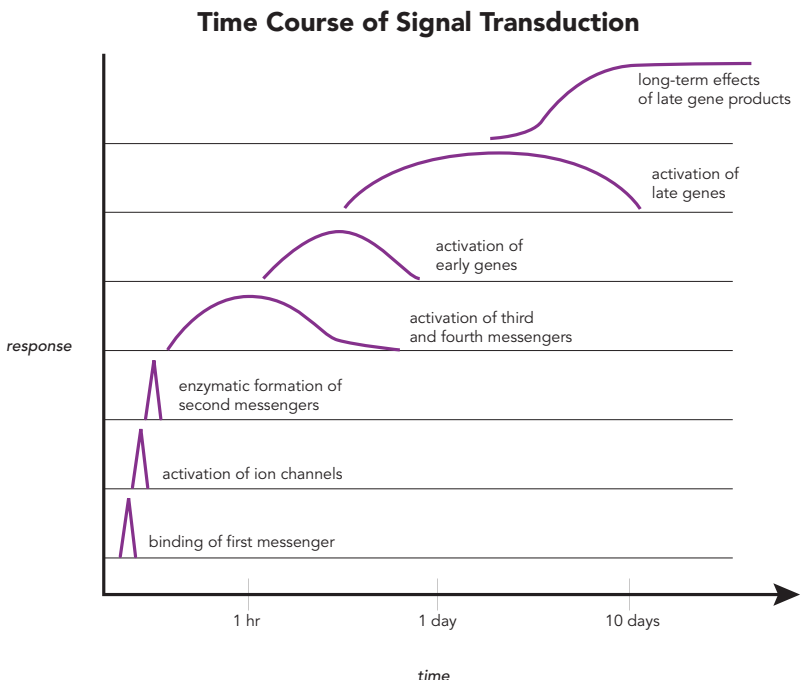


Figure 1-10 Time course of signal transduction. The time course of signal transduction is shown here. The process begins with binding of a first messenger (bottom), which leads to activation of ion channels or enzymatic formation of second messengers. This, in turn, can cause activation of third and fourth messengers, which are often phosphoproteins. If genes are subsequently activated, this leads to the synthesis of new proteins, which can alter the neuron's functions. Once initiated, the functional changes due to protein activation or new protein synthesis can last for at least many days and possibly much longer. Thus, the ultimate effects of signal transduction cascades triggered by chemical neurotransmission are not only delayed but also long-lasting.

chemical activity that gets translated into active fourth messengers able to trigger diverse biological responses, such as gene expression and synaptogenesis (Figure 1-9). Each molecular site within the cascade of transduction of chemical and electrical messages is a potential location for a malfunction associated with a mental illness; it is also a potential target for a psychotropic drug. Thus, the various elements of multiple signal transduction cascades play very important roles in psychopharmacology.

Four of the most important signal transduction cascades in the brain are shown in Figure 1-11. These include G-protein-linked systems, ion-channel-linked systems, hormone-linked systems, and neurotrophin-linked systems. There are many chemical messengers for each of these four critical signal transduction cascades; the G-protein-linked and the ion-channel-linked cascades are triggered by neurotransmitters (Figure 1-11). Many of the psychotropic drugs used in clinical practice today target one of these two signal transduction cascades. Drugs that target the G-protein-linked system are discussed in Chapter 2; drugs that target the ion channel-linked system are discussed in Chapter 3.

Forming a Second Messenger

Each of the four signal transduction cascades (Figure 1-11) passes its message from an extracellular first messenger to an intracellular second messenger.

In the case of G-protein-linked systems, the second messenger is a chemical, but in the case of an ion-channel-linked system, the second messenger can be an ion such as calcium (Figure 1-11). For some hormone-linked systems, a second messenger is formed when the hormone finds its receptor in the cytoplasm and binds to it to form a hormone–nuclear receptor complex (Figure 1-11). For neurotrophins, a complex set of various second messengers exist (Figure 1-11), including proteins that are kinase enzymes with an alphabet soup of complicated names.

The transduction of an extracellular first neurotransmitter from the presynaptic neuron into an intracellular second messenger in the postsynaptic neuron is known in detail for some second-messenger systems, such as for those that are linked to G proteins (Figures 1-12 through 1-15). There are four key elements to this second-messenger system:

- the first-messenger neurotransmitter
- a receptor for the neurotransmitter that belongs to the receptor superfamily in which all have the structure of seven transmembrane regions (designated by the number 7 on the receptor in Figures 1-12 to 1-15)
- a G protein capable of binding both to certain conformations of the neurotransmitter receptor (7) and to an enzyme system (E) that can synthesize the second messenger

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

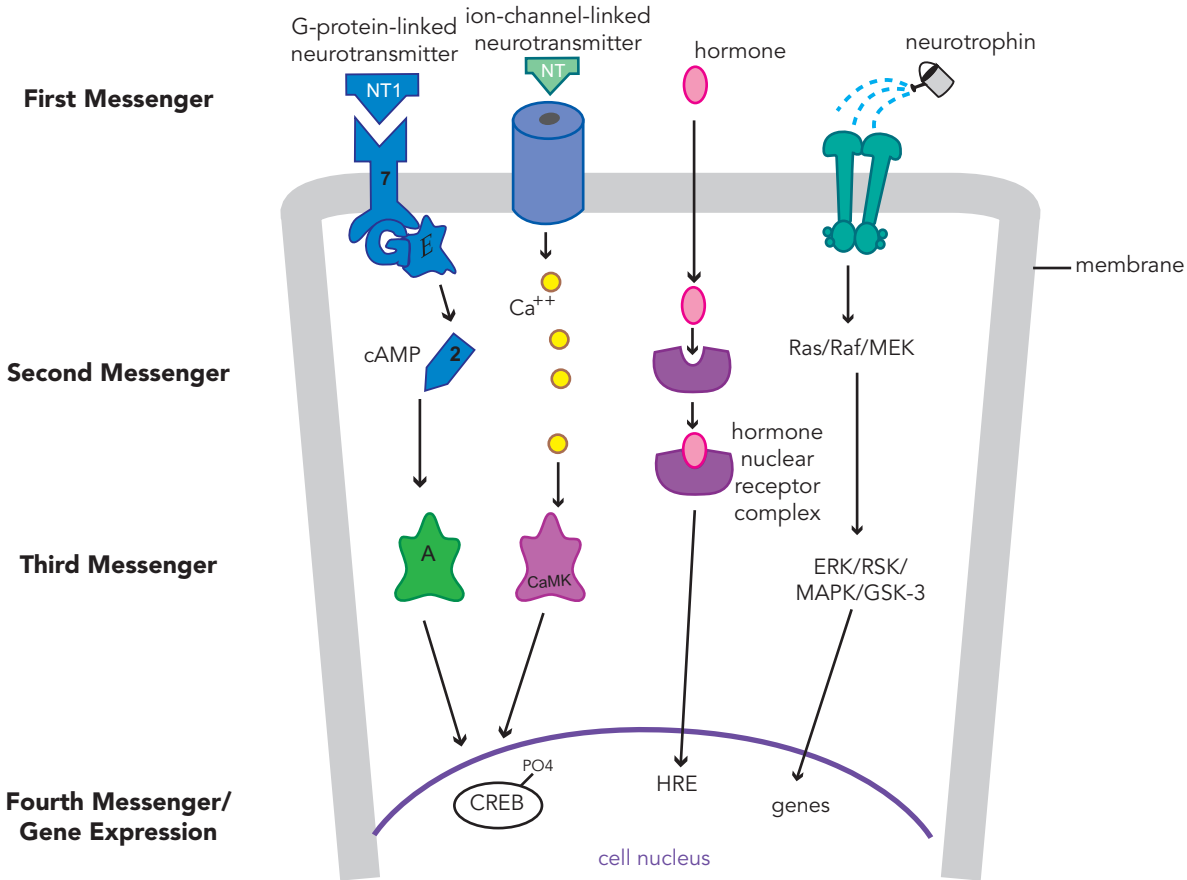


Figure 1-11 **Different signal transduction cascades.** Four of the most important signal transduction cascades in the brain are shown here. These include G-protein-linked systems, ion-channel-linked systems, hormone-linked systems, and neurotrophin-linked systems. Each begins with a different first messenger binding to a unique receptor, leading to activation of very different downstream second, third, and subsequent chemical messengers. Having many different signal transduction cascades allows neurons to respond in amazingly diverse biological ways to a whole array of chemical messaging systems. Neurotransmitters (NTs) activate both the G-protein-linked system and the ion-channel-linked system on the left, and both of these systems activate genes in the cell nucleus by phosphorylating a protein there called cAMP response element-binding protein (CREB). The G-protein-linked system works through a cascade involving cAMP (adenosine monophosphate) and protein kinase A, whereas the ion-channel-linked system works through calcium and its ability to activate a different kinase called calcium/calmodulin kinase (CaMK). Certain hormones, such as estrogen and other steroids, can enter the neuron, find their receptors in the cytoplasm, and bind them to form a hormone–nuclear receptor complex. This complex can then enter the cell nucleus to interact with hormone-response elements (HREs) there to trigger activation of specific genes. Finally, the neurotrophin system on the far right activates a series of kinase enzymes, with a confusing alphabet soup of names, to trigger gene expression, which may control such functions as synaptogenesis and neuronal survival. Ras is a G protein, Raf is a kinase, and the other elements in this cascade are proteins as well (MEK stands for mitogen-activated protein kinase/extracellular signal-regulated kinase; ERK stands for extracellular signal-regulated kinase itself; RSK is ribosomal S6 kinase; MAPK is MAP kinase itself, and GSK-3 is glycogen synthase kinase 3).

- and finally the enzyme system itself for the second messenger (Figures 1-12 through 1-15)

The first step is the neurotransmitter binding to its receptor (Figure 1-13). This changes the conformation of the receptor so it can now fit with the G protein, as indicated by the receptor (7) turning green and its shape changing at the bottom. Next comes the binding of the G protein to this new conformation of the receptor–neurotransmitter complex (Figure 1-14). The two receptors cooperate with each other: namely, the neurotransmitter receptor itself, and the G protein, which can be thought of as another type of receptor associated with the inner membrane of the cell. This cooperation is indicated in Figure 1-14 by the G protein turning green and its conformation changing on the right so it is now capable of binding to an enzyme (E) that synthesizes the second messenger. Finally, the enzyme, in this case adenylate cyclase, binds to the G protein and synthesizes

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

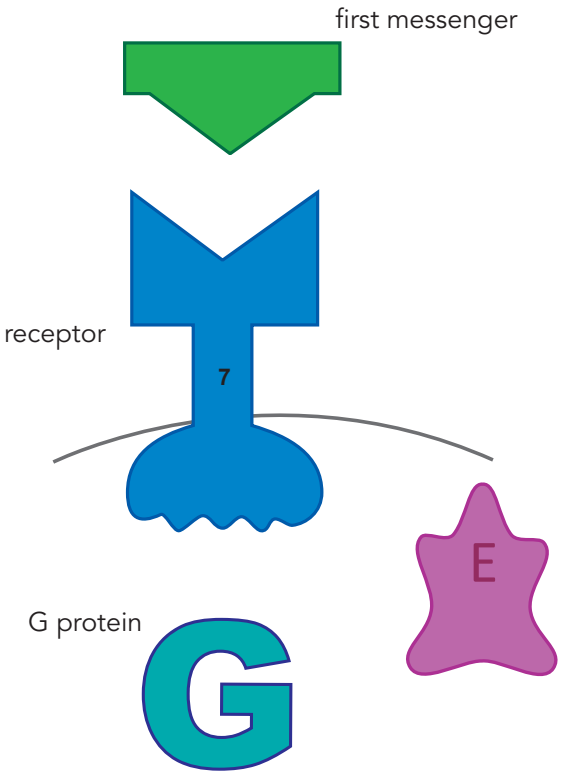


Figure 1-12 Elements of G-protein-linked system. Shown here are the four elements of a G-protein-linked second-messenger system. The first element is the neurotransmitter itself, sometimes also referred to as the first messenger. The second element is the G-protein-linked neurotransmitter receptor, which is a protein with seven transmembrane regions. The third element, a G protein, is a connecting protein. The fourth element of the second-messenger system is an enzyme, which can synthesize a second messenger when activated.

cAMP (cyclic adenosine monophosphate), which serves as second messenger (Figure 1-15). This is indicated in Figure 1-15 by the enzyme turning green and generating cAMP (the icon with number 2 on it).

Beyond the Second Messenger to Phosphoprotein Messengers

Recent research has begun to clarify the complex molecular links between the second messenger and its ultimate effects upon cellular functions. These links are specifically the third, fourth, and subsequent chemical messengers in the signal transduction cascades shown in Figures 1-9, 1-11, 1-16 through 1-30). Each of the four classes of signal transduction cascades shown in Figure 1-11 not only begins with a different first messenger binding to a unique receptor, but this also leads to activation of very different downstream second, third,

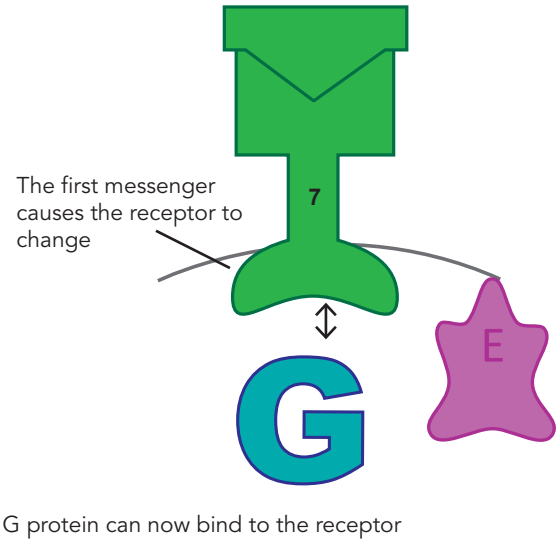


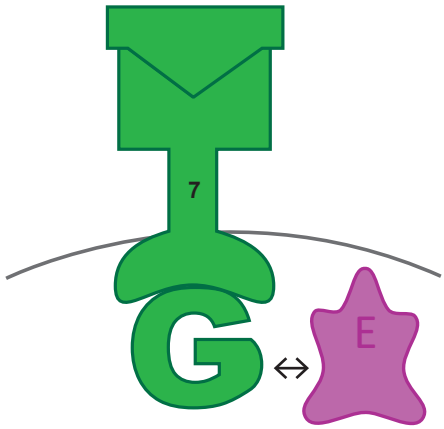
Figure 1-13 First messenger. In this figure, the neurotransmitter has docked into its receptor. The first messenger does its job by transforming the conformation of the receptor so that the receptor can bind to the G protein, indicated here by the receptor turning the same color as the neurotransmitter and changing its shape at the bottom in order to make it capable of binding to the G protein.

and subsequent chemical messengers. Having many different signal transduction cascades allows neurons to respond in amazingly diverse biological ways to a whole array of chemical messaging systems.

What is the ultimate target of signal transduction? There are two major targets of signal transduction: phosphoproteins and genes. Many of the intermediate targets along the way to the gene are phosphoproteins, such as the fourth-messenger phosphoproteins shown in Figures 1-18 and 1-19 that lie dormant in the neuron until signal transduction wakes them up and they can spring into action.

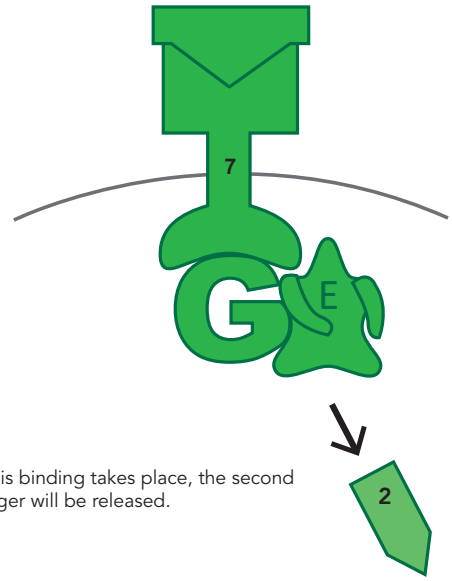
The actions shown in Figure 1-9 on fourth-messenger phosphoproteins as targets of signal transduction can be seen in more detail in Figures 1-16 through 1–19. Thus, one signal transduction pathway can activate a third-messenger kinase through the second-messenger cAMP (Figure 1-16), whereas another signal transduction pathway can activate a third-messenger phosphatase through the second-messenger calcium (Figure 1-17). In the case of kinase activation, two copies of the second messenger target each regulatory unit of dormant or "sleeping" protein kinase (Figure 1-16). When some protein kinases are inactive, they exist in dimers (two copies of the enzyme) while binding to a regulatory unit, thus rendering them in a conformation that is not active.

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press



Once bound to the receptor, the G protein changes shape so it can bind to an enzyme capable of synthesizing a second messenger.

Figure 1-14 G protein. The next stage in producing a second messenger is for the transformed neurotransmitter receptor to bind to the G protein, depicted here by the G protein turning the same color as the neurotransmitter and its receptor. Binding of the binary neurotransmitter-receptor complex to the G protein causes yet another conformational change, this time in the G protein, represented here as a change in the shape of the right-hand side of the G protein. This prepares the G protein to bind to the enzyme capable of synthesizing the second messenger.



Once this binding takes place, the second messenger will be released.

Figure 1-15 Second messenger. The final step in formation of the second messenger is for the ternary complex neurotransmitter–receptor–G protein to bind to a messenger-synthesizing enzyme, depicted here by the enzyme turning the same color as the ternary complex. Once the enzyme binds to this ternary complex, it becomes activated and capable of synthesizing the second messenger. Thus, it is the cooperation of all four elements, wrapped together as a quaternary complex, that leads to the production of the second messenger. Information from the first messenger thus passes to the second messenger through use of receptor–G protein–enzyme intermediaries.

Activating a Third-Messenger Kinase through Cyclic AMP

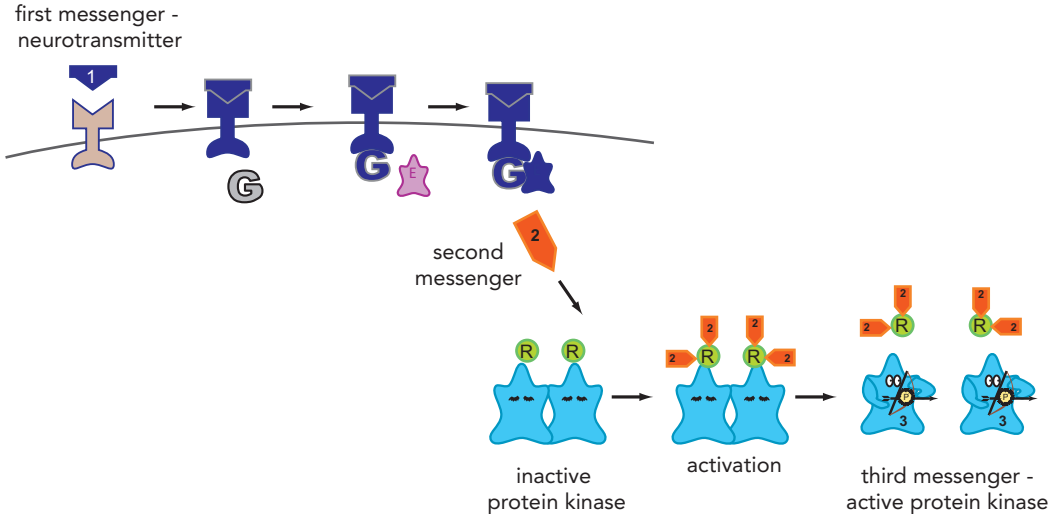


Figure 1-16 Third-messenger protein kinase. This figure illustrates activation of a third-messenger protein kinase through the second-messenger cAMP. Neurotransmitters begin the process of activating genes by producing a second messenger (cAMP), as shown previously in Figures 1-12 through 1-15. Some second messengers activate intracellular enzymes known as protein kinases. This enzyme is shown here as inactive when it is paired with another copy of the enzyme plus two regulatory units (R). In this case, two copies of the second messenger interact with the regulatory units, dissociating them from the protein kinase dimer. This dissociation activates each protein kinase, readying this enzyme to phosphorylate other proteins.

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

Activating a Third-Messenger Phosphatase through Calcium

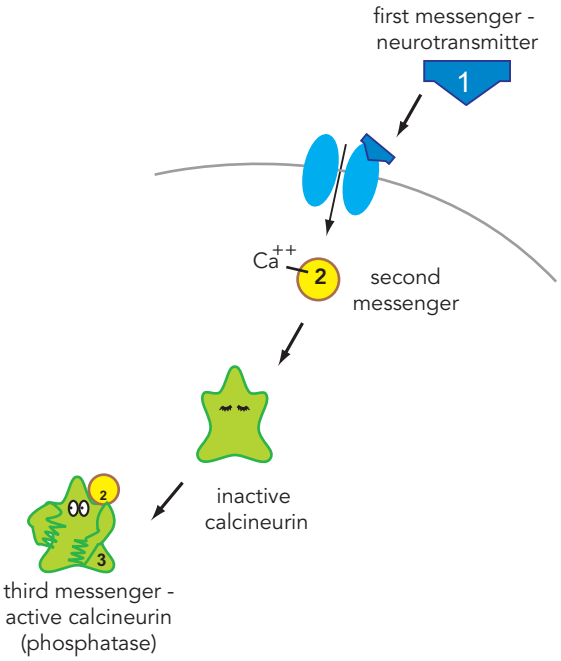


Figure 1-17 Third-messenger phosphatase. This figure illustrates activation of a third-messenger phosphatase through the second-messenger calcium. Shown here is calcium binding to an inactive phosphatase known as calcineurin, thereby activating it and thus readying it to remove phosphates from fourth-messenger phosphoproteins.

In this example, when two copies of cAMP bind to each regulatory unit, the regulatory unit dissociates from the enzyme, and the dimer dissociates into two copies of the enzyme, and the protein kinase is now activated, shown with a bow and arrow ready to shoot phosphate groups into unsuspecting fourth-messenger phosphoproteins (Figure 1-16).

Meanwhile, the nemesis of protein kinase is also forming in Figure 1-17, namely a protein phosphatase. Another first messenger is opening an ion channel here, allowing the second-messenger calcium to enter, which activates the phosphatase enzyme calcineurin. In the presence of calcium, calcineurin becomes activated, shown with scissors ready to rip phosphate groups off fourth-messenger phosphoproteins (Figure 1-17).

The clash between kinase and phosphatase can be seen by comparing what happens in Figures 1-18 and 1-19. In Figure 1-18, the third-messenger kinase is putting phosphates onto various fourth-messenger phosphoproteins such as ligand-gated ion channels, voltage-gated ion channels, and enzymes. In Figure 1-19, the third-messenger phosphatase is taking those phosphates off. Sometimes phosphorylation activates a dormant phosphoprotein; for other phosphoproteins, dephosphorylation can be activating. Activation of fourth-messenger phosphoproteins can change the synthesis of neurotransmitters, alter neurotransmitter release, change the conductance of ions, and generally maintain the chemical neurotransmission apparatus in either a state of readiness or dormancy. The balance between phosphorylation and dephosphorylation of fourth-messenger kinases and phosphatases plays a vital role in regulating many molecules critical to the chemical neurotransmission process.

Beyond the Second Messenger to a Phosphoprotein Cascade Triggering Gene Expression

The ultimate cellular function that neurotransmission often seeks to modify is gene expression, either turning a gene on or turning a gene off. All four signal transduction cascades shown in Figure 1-11 end with the last molecule influencing gene transcription. Both cascades triggered by neurotransmitters are shown acting upon the CREB system, which is responsive to phosphorylation of its regulatory units (Figure 1-11, left). CREB is cAMP response element-binding protein, a transcription factor

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

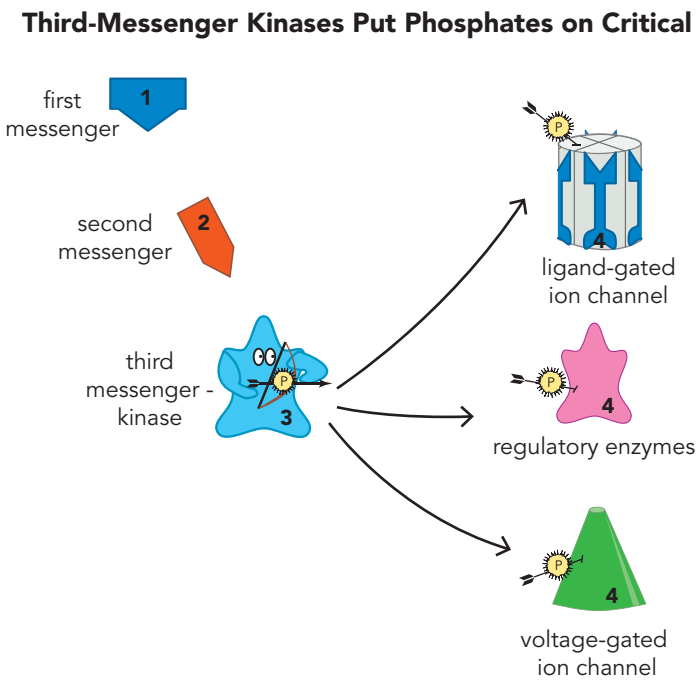


Figure 1-18 **Third-messenger kinase puts phosphates on critical proteins.** Here the activation of a third-messenger kinase adds phosphates to a variety of phosphoproteins, such as ligand-gated ion channels, voltage-gated ion channels, and various regulatory enzymes. Adding a phosphate group to some phosphoproteins activates them; for other proteins, this inactivates them.

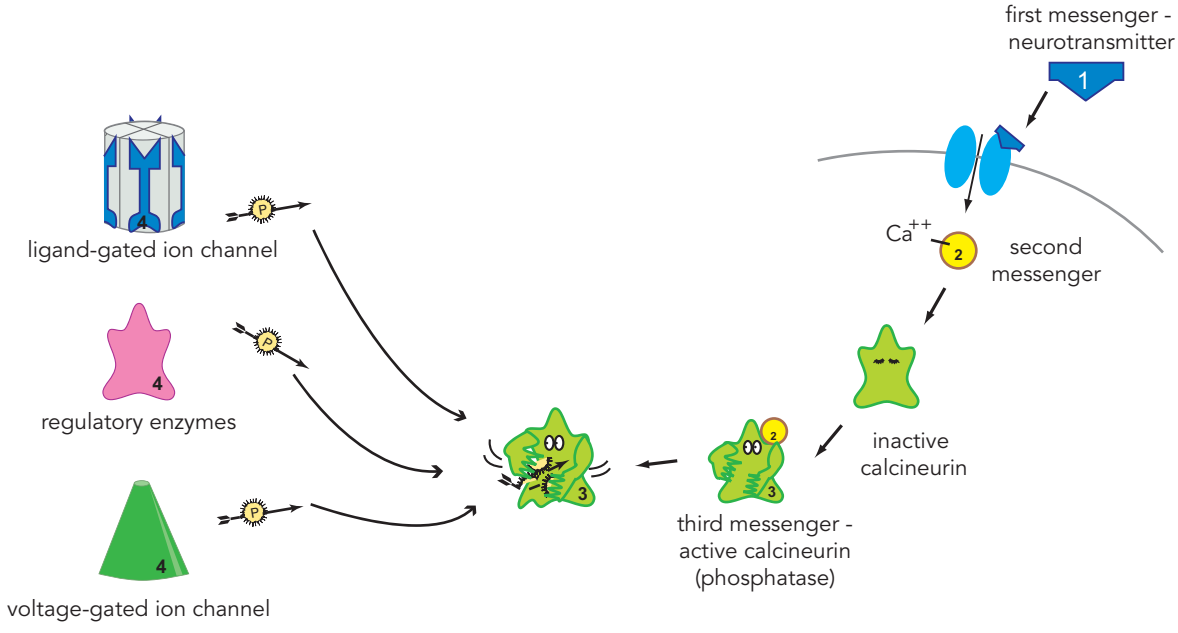


Figure 1-19 **Third-messenger phosphatase removes phosphates from critical proteins.** In contrast to the previous figure, the third messenger here is a phosphatase; this enzyme removes phosphate groups from phosphoproteins such as ligand-gated ion channels, voltage-gated ion channels, and various regulatory enzymes. Removing a phosphate group from some phosphoproteins activates them; for others, it inactivates them.

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

in the cell nucleus capable of activating expression of genes, especially a type of gene known as immediate genes or immediate early genes. When G-protein-linked receptors activate protein kinase A, this activated enzyme can translocate or move into the cell nucleus and stick a phosphate group on CREB, thus activating this transcription factor and causing the nearby gene to become activated. This leads to gene expression, first as RNA and then as the protein coded by the gene.

Interestingly, it is also possible for ion-channel-linked receptors that enhance intracellular second-messenger calcium levels to activate CREB by phosphorylating it. A protein known as calmodulin, which interacts with calcium, can lead to activation of certain kinases called calcium/calmodulin-dependent protein kinases (Figure 1-11). This is an entirely different enzyme than the phosphatase shown in Figures 1-9, 1-17, and 1-19. Here, a kinase and not a phosphatase is activated. When activated, this kinase can translocate into the cell nucleus and, just like the kinase activated by the G-protein system, add a phosphate group to CREB and activate this transcription factor so that gene expression is triggered.

It is important to bear in mind that calcium is thus able to activate both kinases and phosphatases. There is a very rich and sometimes confusing array of kinases and phosphatases, and the net result of calcium action is dependent upon what substrates are activated, because different phosphatases and kinases target very different substrates. Thus, it is important to keep in mind the specific signal transduction cascade under discussion and the specific phosphoproteins acting as messengers in the cascade in order to understand the net effect of various signal transduction cascades. In the case illustrated in Figure 1-11, the G-protein system and the ion-channel system are working together to produce more activated kinases and thus more activation of CREB. However, in Figures 1-9 and 1-16 through 1-19, they are working in opposition.

Genes are also the ultimate target of the hormone signal transduction cascade in Figure 1-11. Some hormones, such as estrogen, thyroid, and cortisol, act at cytoplasmic receptors, bind them, and produce a hormone–nuclear receptor complex that translocates to the cell nucleus, finds elements in the gene that it can influence (called hormone-response elements, or HREs), and then acts as a transcription factor to trigger activation of nearby genes (Figure 1-11).

Finally, a very complicated signal transduction system with terrible sounding names for their downstream signal cascade messengers is activated by neurotrophins and related molecules. Activating this system by first-messenger neurotrophins leads to activation of enzymes that are mostly kinases, one kinase activating another until finally one of them phosphorylates a transcription factor in the cell nucleus and starts transcribing genes (Figure 1-11). Ras is a G protein that activates a cascade of kinases with confusing names. For those who are good sports with an interest in the specifics, this cascade starts with Ras activating Raf, which phosphorylates and activates MEK (MAPK kinase/ERK kinase or mitogen-activated protein kinase kinase/extracellular signal regulated kinase kinase), which activates ERK kinase (extracellular signal-regulated kinase itself), RSK (ribosomal S6 kinase), MAPK (MAP kinase itself), or GSK-3 (glycogen synthase kinase), leading ultimately to changes in gene expression. Confused? It is actually not important to know the names, but to remember the take-away point that neurotrophins trigger an important signal transduction pathway that activates kinase enzyme after kinase enzyme, ultimately changing gene expression. This is worth knowing because this signal transduction pathway may be responsible for the expression of genes that regulate many critical functions of the neuron, such as synaptogenesis and cell survival, as well as the plastic changes that are necessary for learning, memory, and even disease expression in various brain circuits. Both drugs and the environment target gene expression in ways that are just beginning to be understood, including how such actions contribute to the cause of mental illnesses and to the mechanism of action of effective treatments for mental illnesses.

In the meantime, it is mostly important to realize that a very wide variety of genes are targeted by all four of these signal transduction pathways. These range from the genes that make synthetic enzymes for neurotransmitters, to growth factors, cytoskeleton proteins, cellular adhesion proteins, ion channels, receptors, and the intracellular signaling proteins themselves, among many others. When genes are expressed by any of the signal transduction pathways shown in Figure 1-11, this can lead to making more or fewer copies of any of these proteins. Synthesis of such proteins is obviously a critical aspect of the neuron performing its many and varied functions. Numerous diverse biological actions are effected within neurons that alter behaviors in individuals due to gene expression that is triggered by the four major signal transduction cascades. These range widely from neuronal responses such as synaptogenesis, strengthening of

a synapse, neurogenesis, apoptosis, increasing or decreasing the efficiency of information processing in cortical circuits to behavioral responses such as learning, memory, antidepressant responses to antidepressant administration, symptom reduction by psychotherapy, and possibly even the production of a mental illness.

How Neurotransmission Triggers Gene Expression

How does the gene express the protein it codes? The discussion above has shown how the molecular "pony express" of signal transduction has a message encoded with chemical information from the neurotransmitter–receptor complex that is passed along from molecular rider to molecular rider until the message is delivered to the appropriate phosphoprotein mailbox (Figures 1-9 and 1-16 through 1-19) or DNA mailbox in the postsynaptic neuron's genome (Figures 1-11 and 1-20 through 1-30). Since the most powerful way for a neuron to alter its function is to change which genes are being turned on or off, it is important to understand the molecular mechanisms by which neurotransmission regulates gene expression.

How many potential genes can neurotransmission target? It is estimated that the human genome contains approximately *20,000 genes* located within *3 million base pairs* of DNA on 23 chromosomes. Incredibly, however, genes only occupy a few percent of this DNA. The other 96% used to be called "junk" DNA since it does not code proteins, but it is now known that these sections of DNA are critical for structure and for regulating whether or not a gene is expressed or is silent. It is not just the number of genes we have, it is whether and when and how often and under which circumstances they are expressed that seems to be the important factor in regulating neuronal function. These same factors of gene expression are now thought to also underlie the actions of psychopharmacological drugs and the mechanisms of psychiatric disorders within the central nervous system.

Molecular Mechanism of Gene Expression

Chemical neurotransmission converts receptor occupancy by a neurotransmitter into the creation of third, fourth, and subsequent messengers that eventually activate transcription factors that turn on genes (Figures 1-20 through 1-30). Most genes have two regions, a *coding region* and a *regulatory region* with enhancers and promoters of gene transcription (i.e., DNA being transcribed into RNA) (Figure 1-20). The coding region of DNA is the direct template for making its corresponding RNA. This DNA is "transcribed" into its RNA with the help of an enzyme called *RNA polymerase*. However, RNA polymerase must be activated, or it won't work.

Luckily, the regulatory region of the gene can make this happen. It has an *enhancer element* and a *promotor element* (Figure 1-20), which can initiate gene expression with the help of transcription factors (Figure 1-21). Transcription factors themselves can be activated when they are phosphorylated, which allows them to bind to the regulatory region of the gene (Figure 1-21). This in turn activates RNA polymerase and off we go with the coding part of the gene *transcribing* itself into its messenger RNA (mRNA) (Figure 1-22). Once transcribed, of course, this messenger RNA goes on to *translate* itself into the corresponding protein (Figure 1-22). However, there is a great deal of RNA that never gets translated into proteins and instead exerts regulatory functions as explained below.

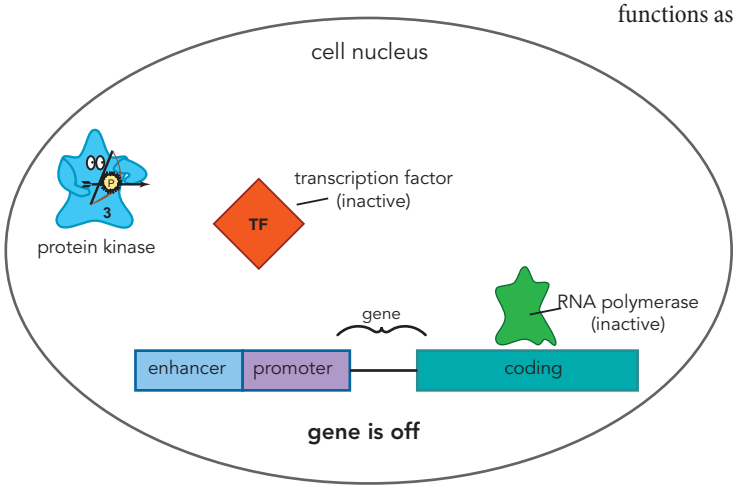


Figure 1-20 Activation of a gene, part 1: gene is off. The elements of gene activation shown here include the enzyme protein kinase; a transcription factor, a type of protein that can activate a gene; RNA polymerase, the enzyme that synthesizes RNA from DNA when the gene is transcribed; the regulatory regions of DNA, such as enhancer and promoter areas; and finally the gene itself. This particular gene is off because the transcription factor has not yet been activated. The DNA for this gene contains both a regulatory region and a coding region. The regulatory region has both an enhancer element and a promoter element, which can initiate gene expression when they interact with activated transcription factors. The coding region is directly transcribed into its corresponding RNA once the gene is activated.

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

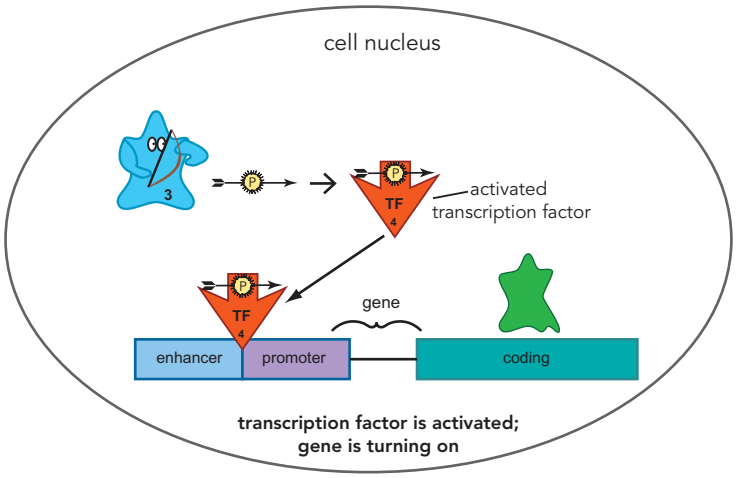


Figure 1-21 Activation of a gene, part 2: gene turns on. The transcription factor is now activated because it has been phosphorylated by protein kinase, allowing it to bind to the regulatory region of the gene.

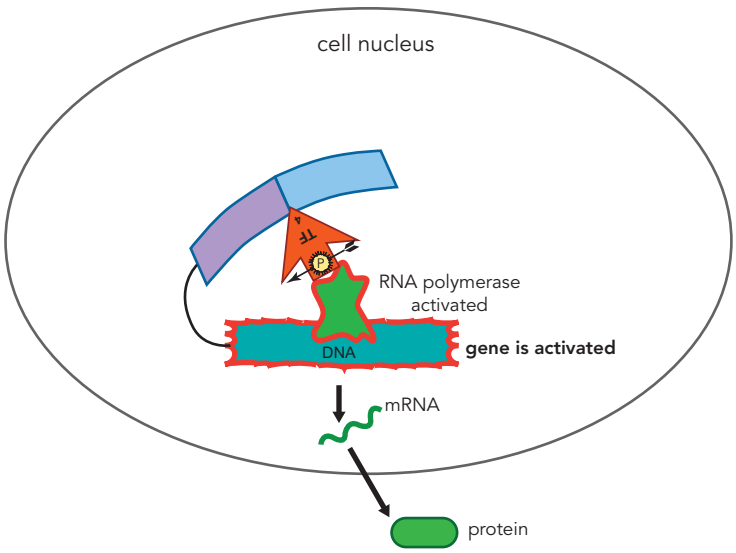


Figure 1-22 Activation of a gene, part 3: gene product. The gene itself is now activated because the transcription factor has bound to the regulatory region of the gene, in turn activating the enzyme RNA polymerase. Thus, the gene is transcribed into messenger RNA (mRNA), which in turn is translated into its corresponding protein. This protein is thus the product of activation of this particular gene.

Third Messenger Activating a Transcription Factor for an Early Gene

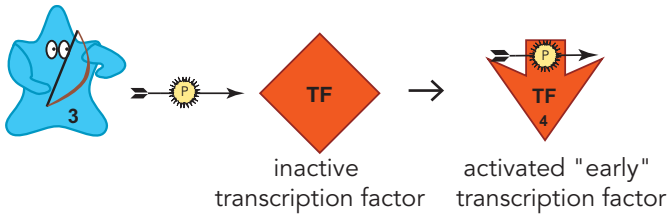


Figure 1-23 Immediate early gene. Some genes are known as immediate early genes. Shown here is a third-messenger protein kinase enzyme activating a transcription factor, or fourth messenger, capable of activating, in turn, an early gene.

Some genes are known as immediate early genes (Figure 1-23). They have weird names such as *cJun* and *cFos* (Figures 1-24 and 1-25) and belong to a family called "leucine zippers" (Figure 1-25). These immediate early genes function as rapid responders to the neurotransmitter's input, like the special ops troops sent into combat quickly and ahead of the full army. Such rapid deployment forces of immediate early genes

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

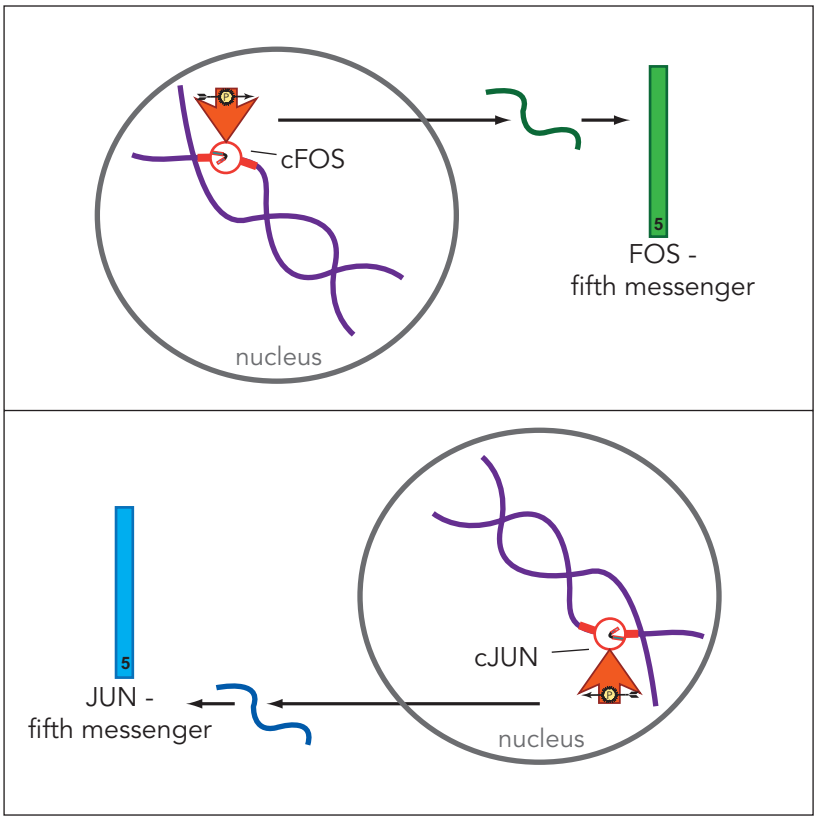


Figure 1-24 Early genes activate late genes, part 1. In the top panel, a transcription factor is activating the immediate early gene *cFos* and producing the protein product Fos. While the *cFos* gene is being activated, another immediate early gene, *cJun*, is being simultaneously activated and producing its protein, Jun, as shown in the bottom panel. Fos and Jun can be thought of as fifth messengers.

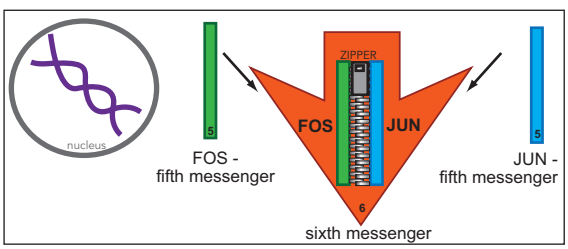


Figure 1-25 Early genes activate late genes, part 2. Once Fos and Jun proteins are synthesized, they can collaborate as partners and produce a Fos–Jun combination protein, which now acts as a sixth-messenger transcription factor for late genes.

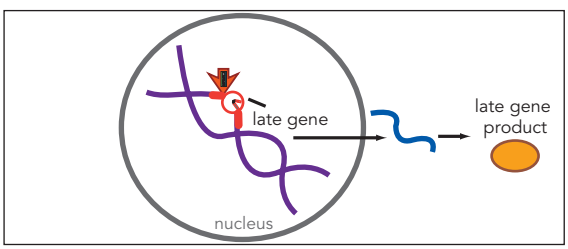


Figure 1-26 Early genes activate late genes, part 3. The Fos-Jun transcription factor belongs to a family of proteins called leucine zippers. The leucine zipper transcription factor formed by the products of the activated early genes *cFos* and *cJun* now returns to the genome and finds another gene. Since this gene is being activated later than the others, it is called a late gene. Thus, early genes activate late genes when the products of early genes are themselves transcription factors. The product of the late gene can be any protein the neuron needs, such as an enzyme, a transport factor, or a growth factor.

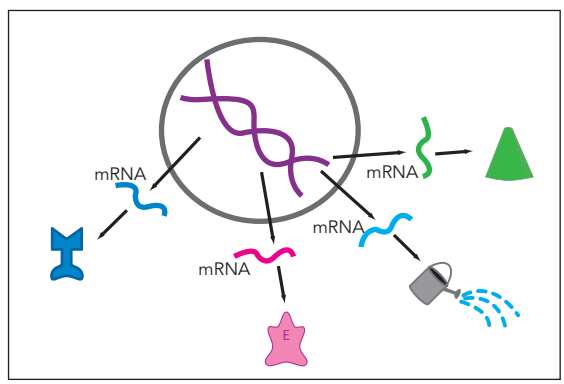


Figure 1-27 Examples of late gene activation. A receptor, an enzyme, a neurotrophic growth factor, and an ion channel are all being expressed owing to activation of their respective genes. Such gene products go on to modify neuronal function for many hours or days.

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

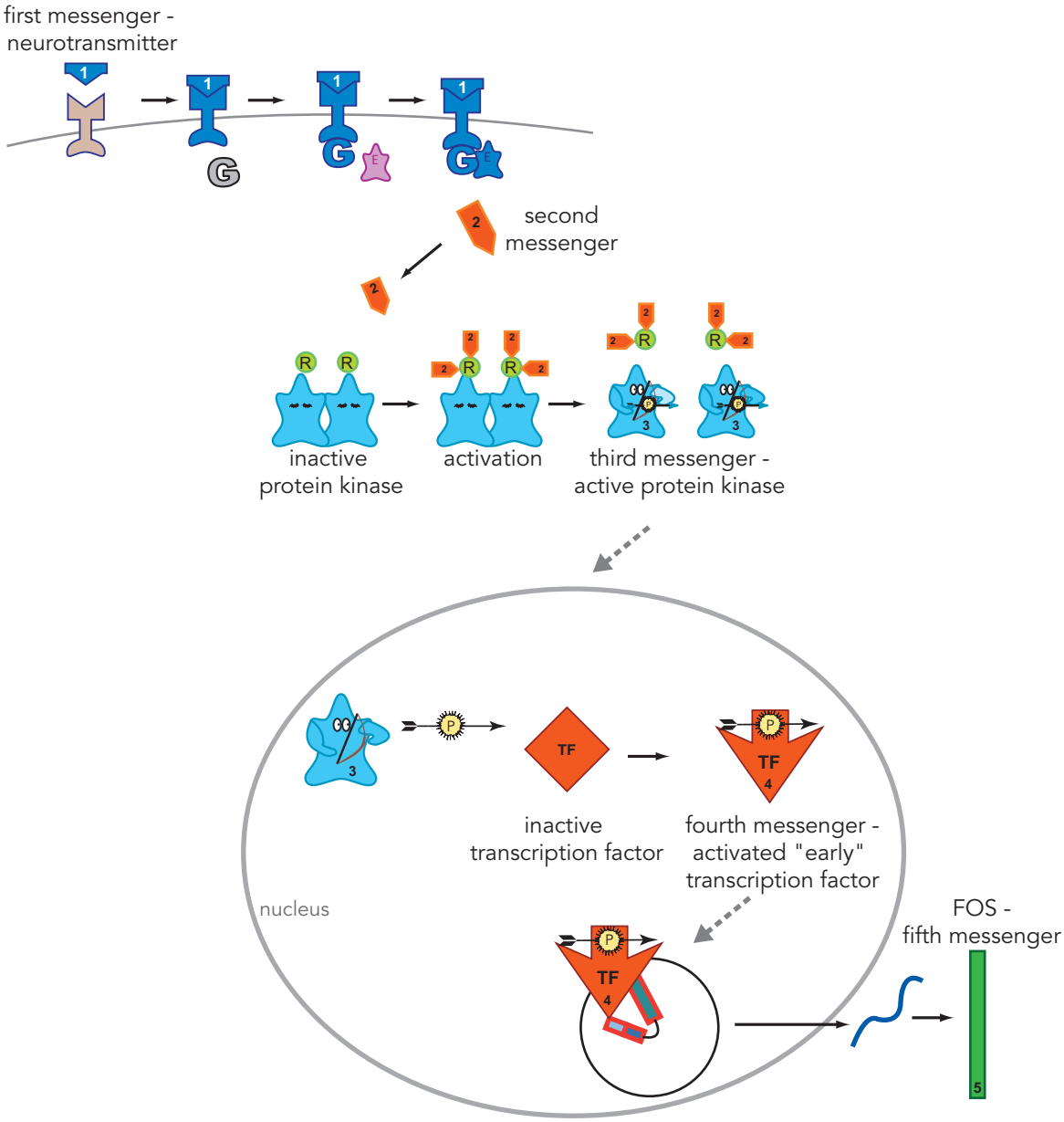


Figure 1-28 Gene regulation by neurotransmitters. This figure summarizes gene regulation by neurotransmitters, from first-messenger extracellular neurotransmitter to intracellular second messenger, to third-messenger protein kinase, to fourth-messenger transcription factor, to fifth-messenger protein, which is the gene product of an early gene.

are thus the first to respond to the neurotransmission signal by making the proteins they encode. In this example, it is Jun and Fos proteins coming from *cJun* and *cFos* genes (Figure 1-24). These are nuclear proteins; that is, they live and work in the nucleus. They get started within 15 minutes of receiving a neurotransmission, but only last for a half hour to an hour (Figure 1-10).

When Jun and Fos team up, they form a leucine zipper type of transcription factor (Figure 1-25), which in turn activates many kinds of later-onset genes (Figures 1-26, 1-27, 1-29). Thus, Fos and Jun serve to wake up the much larger army of inactive genes. Which individual "late" soldier genes are so drafted to active gene duty depends upon a number of factors, not the least of which is which neurotransmitter is sending the message, how frequently

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

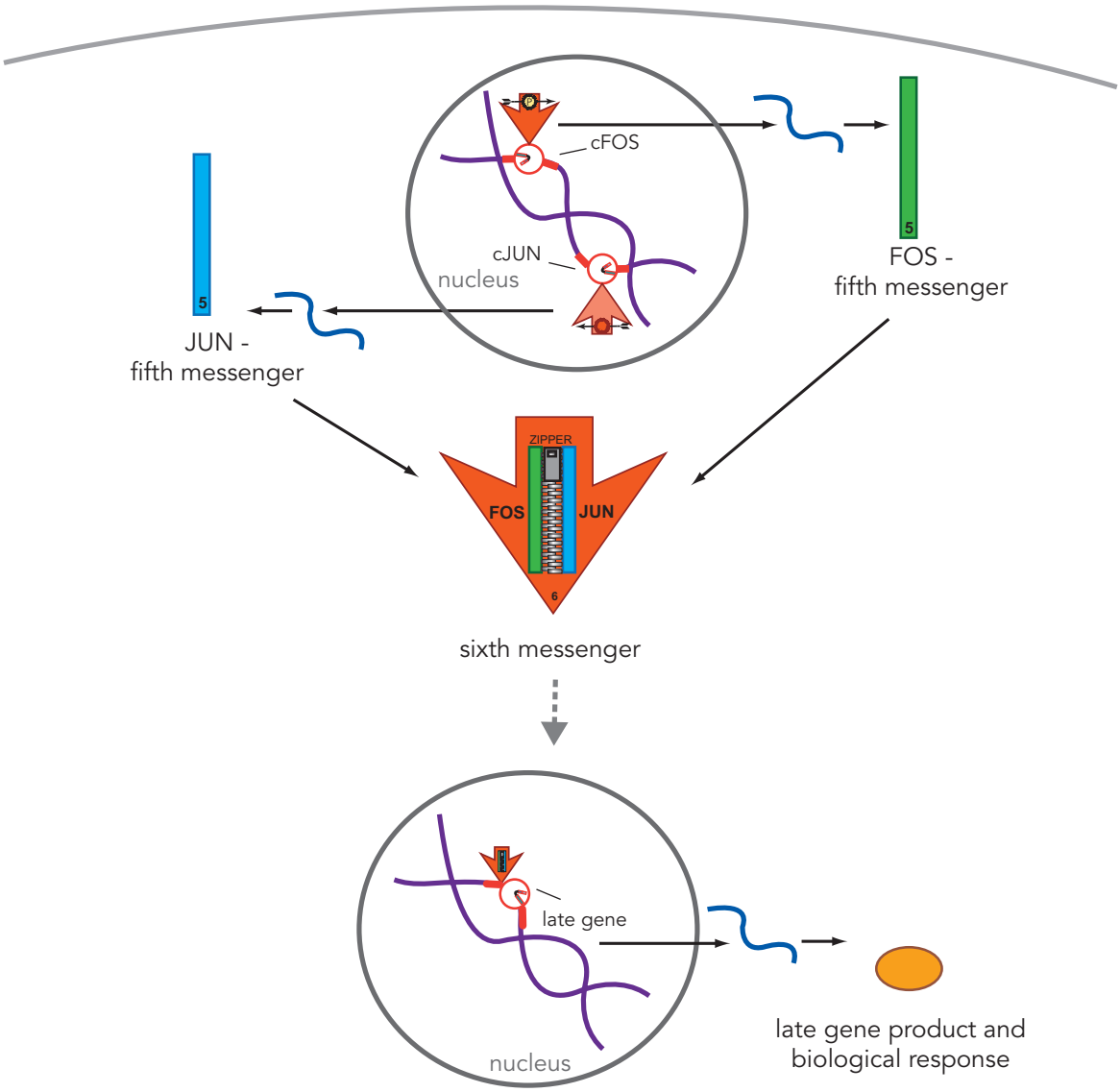


Figure 1-29 **Activating a late gene.** This figure summarizes the process of activating a late gene. At the top, immediate early genes *cFos* and *cJun* are expressed and their fifth-messenger protein products Fos and Jun are formed. Next, a transcription factor, namely a leucine zipper, is created by the cooperation of Fos and Jun together, combining to form the sixth messenger. Finally, this transcription factor goes on to activate a late gene, resulting in the expression of its own gene product and the biological response triggered by that late gene product.

it is sending the message, and whether it is working in concert or in opposition with other neurotransmitters talking to other parts of the same neuron at the same time. When Fos and Jun partner together to form a leucine zipper type of transcription factor, this can lead to the activation of genes to make anything you can think of, from enzymes to receptors to structural proteins (see Figure 1-27).

In summary, one can trace the events from the neurotransmitting first messenger, through gene transcription (Figures 1-9, 1-11, 1-28, and 1-29). Once the second-messenger cAMP is formed from its first-messenger neurotransmitter (Figure 1-28), it can interact with a protein kinase third messenger. cAMP binds to the inactive or sleeping version of this enzyme, wakes it up, and thereby activates protein kinase. Once awakened,

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

the protein kinase third messenger's job is to activate transcription factors by phosphorylating them (Figure 1-28). It does this by traveling straight to the cell nucleus and finding a sleeping transcription factor. By sticking a phosphate onto the transcription factor, protein kinase is able to "wake up" that transcription factor and form a fourth messenger (Figure 1-28). Once a transcription factor is aroused, it will bind to genes and cause protein synthesis, in this case, the product of an immediate early gene, and this functions as a fifth messenger. Two such gene products bind together to form yet another activated transcription factor, and this is the sixth messenger (Figure 1-29). Finally, the sixth messenger causes the expression of a late gene product, which could be thought of as a seventh-messenger protein product of the activated gene. This late gene product then mediates some biological response important to the functioning of the neuron.

Of course, neurotransmitter-induced molecular cascades into the cell nucleus lead to changes not only in the synthesis of its own receptors, but also in that of many other important postsynaptic proteins, including enzymes and receptors for other neurotransmitters. If such changes in genetic expression lead to changes in connections and in the functions that these connections perform, it is easy to understand how genes can *modify behavior*. The details of nerve functioning – and thus the behavior derived from this nerve functioning – are controlled by genes and the products they produce. Since mental processes and the behaviors they cause come from the connections between neurons in the brain, genes therefore exert significant control over behavior. But can behavior modify genes? Learning as well as experiences from the environment can indeed alter which genes are expressed and thus can give rise to changes in neuronal connections. In this way, human experiences, education, and even psychotherapy may change the expression of genes that alter the distribution and "strength" of specific synaptic connections. This in turn may produce long-term changes in behavior caused by the original experience and mediated by the genetic changes triggered by that original experience. Thus, genes modify behavior and behavior modifies genes. Genes do not directly regulate neuronal functioning. Rather, they directly regulate the proteins which create neuronal functioning. Changes in function have to wait until the changes in protein synthesis occur, and the events which they cause start to happen.

EPIGENETICS

Genetics is the DNA code for what a cell can transcribe into specific types of RNA or translate into specific proteins. However, just because there are about 20,000 genes in the human genome, it does not mean that every gene is expressed, even in the brain. Epigenetics is a parallel system that determines whether any given gene is actually made into its specific RNA and protein, or if it is instead ignored or silenced. If the genome is a lexicon of all protein "words," then the epigenome is a "story" resulting from arranging the "words" into a coherent tale. The genomic lexicon of all potential proteins is the same in every one of the 100+ billion neurons in the brain, and indeed is the same in all of the 200+ types of cells in the body. So, the plot of how a normal neuron becomes a malfunctioning neuron in a psychiatric disorder, as well as how a neuron becomes a neuron instead of a liver cell, is the selection of which specific genes are expressed or silenced. In addition, malfunctioning neurons are impacted by inherited genes that have abnormal nucleotide sequences, which if expressed contribute to mental disorders. Thus, the story of the brain depends not only upon which genes are inherited but also whether any abnormal genes are expressed or even whether normal genes are expressed when they should be silent or silenced when they should be expressed. Neurotransmission, genes themselves, drugs, and the environment all regulate which genes are expressed or silenced, and thus all affect whether the story of the brain is a compelling narrative such as learning and memory, a regrettable tragedy such as drug abuse, stress reactions, and psychiatric disorders, or therapeutic improvement of a psychiatric disorder by medications or psychotherapy.

What Are the Molecular Mechanisms of Epigenetics?

Epigenetic mechanisms turn genes on and off by modifying the structure of chromatin in the cell nucleus (Figure 1-30). The character of a cell is fundamentally determined by its chromatin, a substance composed of nucleosomes (Figure 1-30). Nucleosomes are an octet of proteins called histones around which DNA is wrapped (Figure 1-30). Epigenetic control over whether a gene is read (i.e., expressed) or is not read (i.e., silenced), is done by modifying the structure of chromatin. Chemical modifications that can do this include not only methylation, but also acetylation, phosphorylation, and others, and these processes are regulated by neurotransmission, drugs, and the environment (Figure 1-30). For example, when DNA or histones are

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

methylated, this compacts the chromatin and acts to close off access of molecular transcription factors to the promoter regions of DNA, with the consequence that the gene in this region is silenced, and not expressed, so no RNA or protein is manufactured (Figure 1-30). Silenced DNA means molecular features that are not part of a given cell's personality.

Histones are methylated by enzymes called histone methyltransferases, and this is reversed by enzymes called histone demethylases (Figure 1-30). Methylation of histones can silence genes whereas demethylation of histones can thus activate genes. DNA can also be methylated and this, too, silences genes. Demethylation of DNA reverses this. Methylation of DNA is regulated by DNA methyltransferase (DNMT) enzymes, and demethylation of DNA by DNA demethylase enzymes (Figure 1-30). There are many forms of methyltransferase enzymes, and they all tag their substrates with methyl groups donated from L-methylfolate via S-adenosyl-methionine (SAMe) (Figure 1-30). When neurotransmission, drugs, or the environment impact methylation, for example, this regulates whether genes are epigenetically silenced or expressed.

Methylation of DNA can eventually lead to deacetylation of histones as well, by activating enzymes called histone deacetylases (HDACs). Deacetylation of histones also has a silencing action on gene expression (Figure 1-30). Methylation and deacetylation compress chromatin, as though a molecular gate has been closed, and thus transcription factors that activate genes cannot get access to their promoter regions, and thus the genes are silenced and not transcribed into RNA or translated into proteins (Figure 1-30). On the other hand, demethylation and acetylation do just the opposite: they decompress chromatin as though a molecular gate has been opened, and thus transcription factors can get to the promoter regions of genes, and do activate them (Figure 1-30). Activated genes thus become part of the molecular personality of a given cell.

How Epigenetics Maintains or Changes the Status Quo

Some enzymes try to maintain the status quo of a cell, enzymes such as DNMT1 (DNA methyltransferase 1), which maintain the methylation of specific areas of DNA and keep various genes quiet for a lifetime. For example, this process keeps a neuron always a neuron, and a liver cell always a liver cell, including when a cell divides into another one. Presumably methylation is maintained at genes that one cell does not need, even though another cell type might.

It used to be thought that, once a cell differentiated, the epigenetic pattern of gene activation and gene silencing remained stable for the lifetime of that cell. Now, however, it is known that there are various circumstances in which epigenetics may change in mature, differentiated neurons. Although the initial epigenetic pattern of a neuron is indeed set during neurodevelopment to give each neuron its own lifelong "personality," it now appears that the storyline of some neurons is that they respond to their narrative experiences throughout life with a changing character arc, thus causing de novo alterations in their epigenome. Depending upon what happens to a neuron (such as experiencing child abuse, adult stress, dietary deficiencies, productive new encounters, psychotherapy, drugs of abuse, or psychotropic therapeutic medications), it now seems that previously silenced genes can become activated and/or previously active genes can become silenced (Figure 1-30). When this happens, both favorable and unfavorable developments can occur in the character of neurons. Favorable epigenetic mechanisms may be triggered in order for one to learn (e.g., spatial memory formation) or to experience the therapeutic actions of psychopharmacological agents. On the other hand, unfavorable epigenetic mechanisms may be triggered in order for one to become addicted to drugs of abuse, or to experience various forms of "abnormal learning," such as when one develops fear conditioning, an anxiety disorder, or a chronic pain condition.

How these epigenetic mechanisms arrive at the scene of the crime remains a compelling neurobiological and psychiatric mystery. Nevertheless, a legion of scientific detectives is working these cases and is beginning to show how epigenetic mechanisms are mediators of psychiatric disorders. There is also the possibility that epigenetic mechanisms can be harnessed to treat addictions, extinguish fear, prevent the development of chronic pain states, and maybe even prevent disease progression of psychiatric disorders such as schizophrenia by identifying high-risk individuals before the "plot thickens" and the disorder is irreversibly established and relentlessly marches on to an unwanted destiny.

One of the mechanisms for changing the status quo of epigenomic patterns in a mature cell is via de novo DNA methylation by a type of DNMT enzyme known as DNMT2 or DNMT3 (Figure 1-30). These enzymes target neuronal genes for silencing that were previously active in a mature neuron. Of course, deacetylation of histones near previously active genes would do the same thing, namely silence them, and this is mediated

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

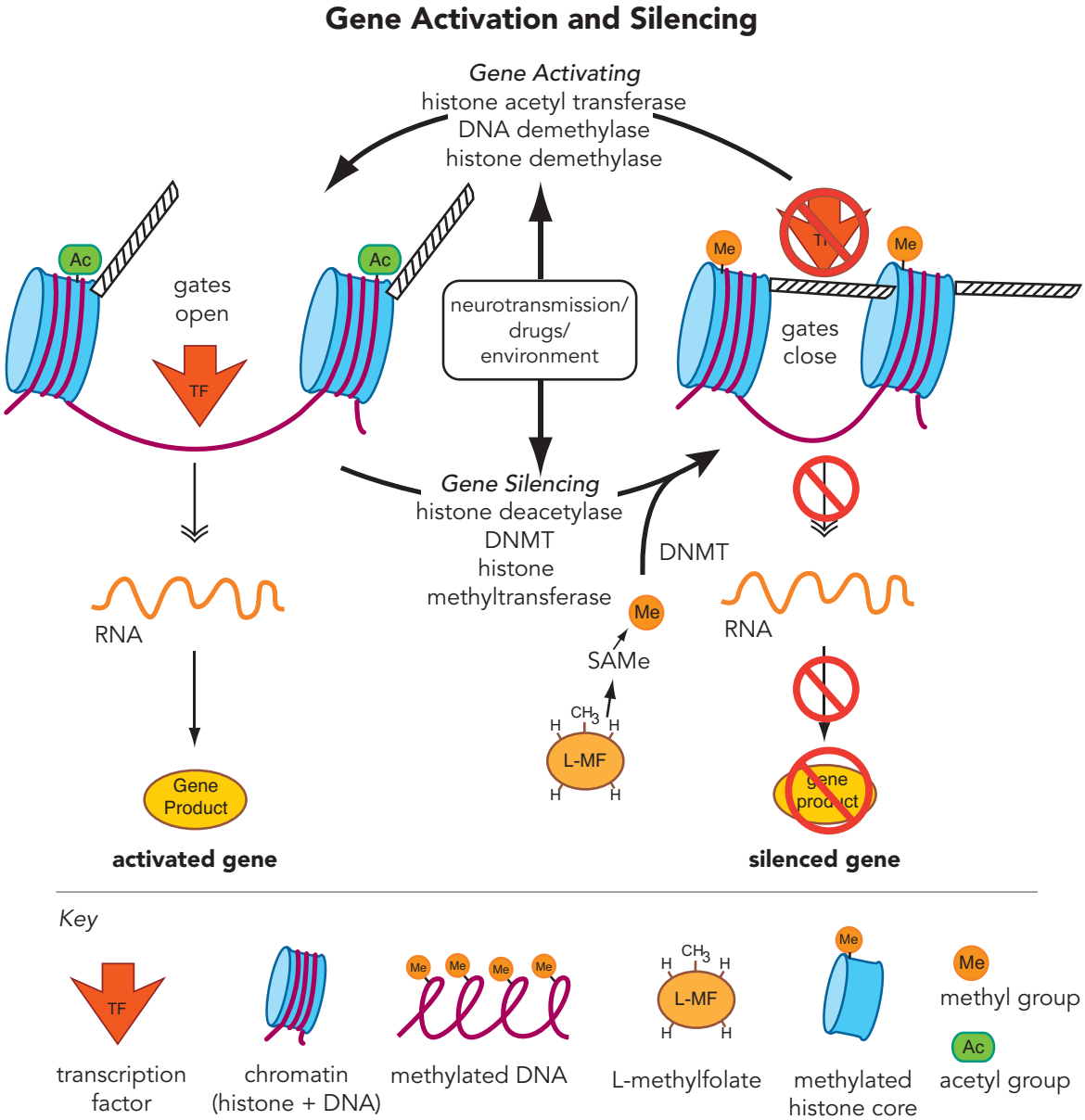


Figure 1-30 Gene activation and silencing. Molecular gates are opened by acetylation and/or demethylation of histones, allowing transcription factors access to genes, thus activating them. Molecular gates are closed by deacetylation and/or methylation provided by the methyl donor SAMe derived from L-methylfolate. This prevents access of transcription factors to genes, thus silencing them. Ac = acetyl; Me = methyl; DNMT = DNA methyltransferase; TF = transcription factor; SAMe = S-adenosyl-methionine; L-MF = L-methylfolate.

by HDACs. In reverse, demethylation or acetylation of genes both activate genes that were previously silent. The real question is how does a neuron know which genes among its thousands to silence or activate in response to the environment, including stress, drugs, and diet? How might this go wrong when a psychiatric disorder develops? This part of the story remains a twisted mystery but some very interesting detective work has already been done by various investigators who hope to understand how some neuronal stories evolve into psychiatric tragedies. These investigations may set the stage for rewriting the narrative of various psychiatric disorders by

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

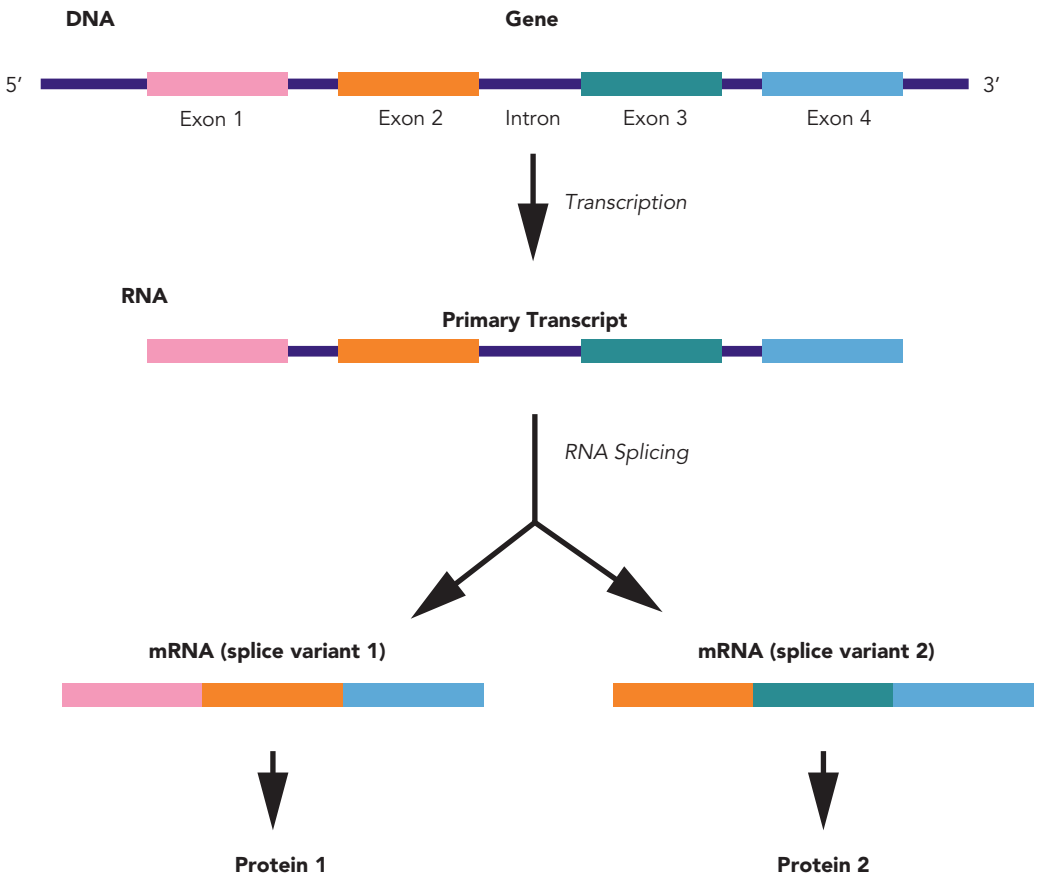


Figure 1-31 **Alternative splicing.** When DNA is transcribed into messenger RNA (mRNA), this is called the primary transcript. The primary transcript can then be translated into a protein; however, sometimes an intermediary step occurs in which the mRNA is spliced, with certain sections reorganized or removed outright. This means that one gene can give rise to more than one protein.

therapeutically altering the epigenetics of key neuronal characters so that the story has a happy ending.

A BRIEF WORD ABOUT RNA

Alternative Splicing

As mentioned above, the RNA that encodes our 20,000 genes is called messenger RNA (mRNA) and serves as an intermediate between DNA and protein. Although it might seem as if our 20,000 genes would make only 20,000 proteins, that is not so. It turns out that developing mRNA into protein is a similar process as when an old-fashioned movie producer makes cinema. That is, mRNA records the action from DNA just as the movie studio faithfully develops the film exactly as initially recorded. In the case of DNA transcription, this "first draft" is called the primary transcript (Figure 1-31). However, just as the raw footage from a movie shoot is not "translated" directly into a motion picture, in many cases, the "raw" mRNA is also not immediately translated into a protein. Now comes the interesting part: editing. It turns out that mRNA can be "spliced," much like a movie producer edits and splices movie film once the live shoot is over, organizing the splices into different sequences and leaving some on the cutting-room floor. For spliced mRNA, these sections won't be translated into protein (Figure 1-31). This "alternative splicing" means that one gene can give rise to many proteins (Figure 1-31), just like a movie can have different endings or be edited into a short trailer. Thus, thanks in part to RNA editing, the true molecular diversity of the brain is notably greater than our 20,000 genes.

RNA Interference

There are forms of RNA other than mRNA that are now known to exist and that do not code for protein

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

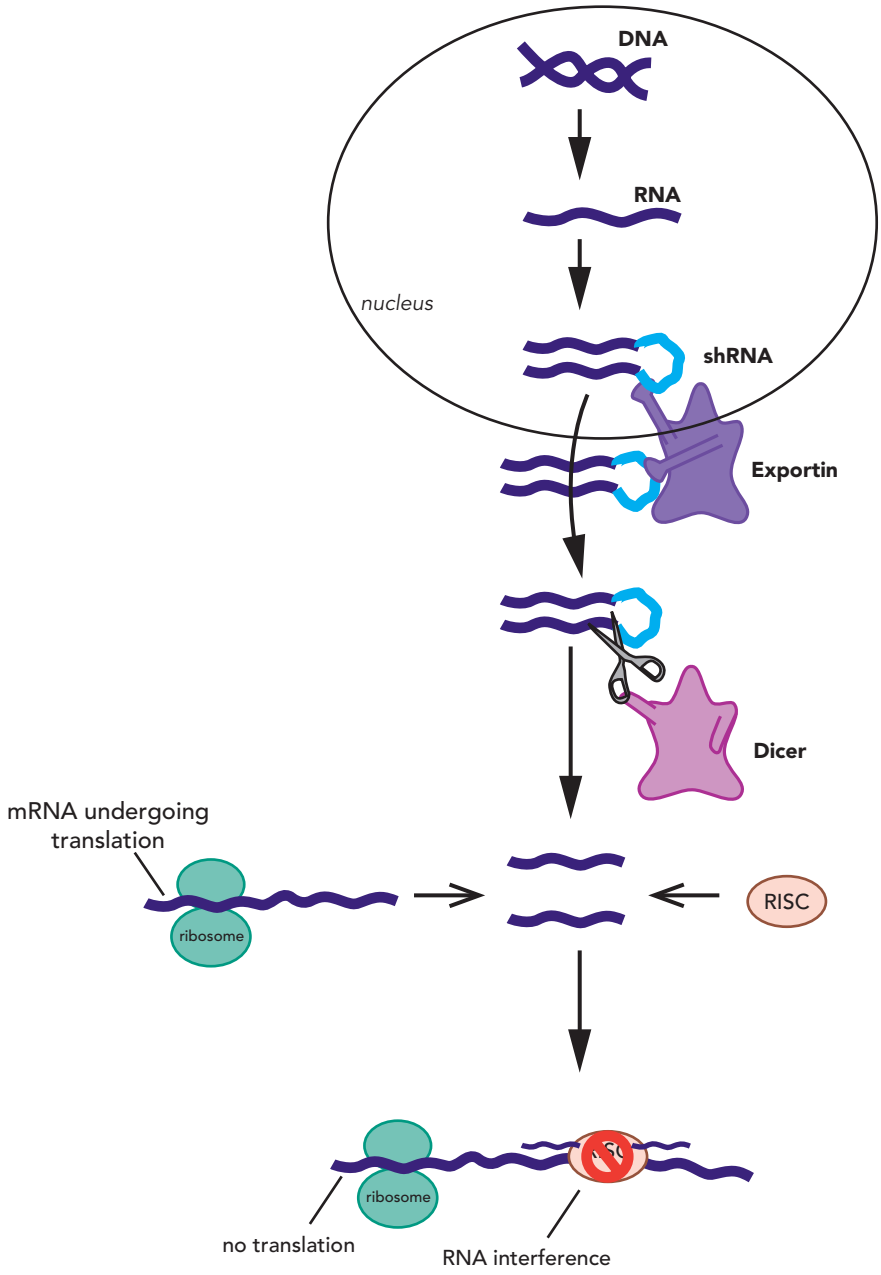


Figure 1-32 **RNA interference.** Some forms of RNA do not code for protein synthesis, and instead have regulatory functions. As shown here, small hairpin RNA (shRNA) is transcribed from DNA but is not translated into protein. Instead, it forms hairpin loops and is exported into the cytoplasm by the enzyme exportin, where it is then chopped into pieces by the enzyme dicer. The small pieces bind to a protein complex called RISC, which in turn binds to mRNA and inhibits protein synthesis.

synthesis; instead they have direct regulatory functions. These include ribosomal RNA (rRNA), transfer RNA (tRNA), and small nuclear RNA (snRNA), along with a large number of other noncoding RNAs (e.g., small hairpin RNAs because they are shaped like a hairpin, sometimes also called microRNA [miRNA]; interference RNA [iRNA]; and small interfering RNA [siRNA]. When miRNAs are transcribed from DNA, they do not go on to be translated into proteins. Instead, they form hairpin loops and are then exported to the cytoplasm by the enzyme exportin, where they are chopped into pieces by an enzyme called "dicer" (Figure 1-32). Small pieces of iRNA then bind to a protein complex called RISC, which binds in turn to mRNA to inhibit protein synthesis (Figure 1-32). So, forms of RNA can lead both to protein synthesis and to blocking protein synthesis.

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press

Future therapeutics may be able to utilize iRNAs to inhibit protein synthesis in genetic disorders, such as Huntington's disease.

SUMMARY

The reader should now appreciate that chemical neurotransmission is the foundation of psychopharmacology. There are many neurotransmitters, and all neurons receive input from a multitude of neurotransmitters in classic presynaptic to postsynaptic asymmetrical neurotransmission. Presynaptic to postsynaptic neurotransmissions at the brain's trillion synapses are key to chemical neurotransmission, but some neurotransmission is retrograde from postsynaptic neuron to presynaptic neuron, and other types of neurotransmission, such as volume neurotransmission, do not require a synapse at all.

The reader should also have an appreciation for elegant if complex molecular cascades precipitated by a neurotransmitter, with molecule-by-molecule transfer of that transmitted message inside the neuron receiving that message, eventually altering the biochemical machinery of that cell in order to carry out the message that was sent to it. Thus, the function of chemical neurotransmission is not so much to have a presynaptic neurotransmitter communicate with its postsynaptic receptors, but to have a *presynaptic genome converse with a postsynaptic genome*: DNA to DNA, presynaptic "command center" to postsynaptic "command center" and back.

The message of chemical neurotransmission is transferred via three sequential "molecular pony express" routes: (1) a presynaptic neurotransmitter synthesis route from presynaptic genome to the synthesis and packaging of neurotransmitter and supporting enzymes and receptors; (2) a postsynaptic route from receptor occupancy through second messengers all the way to the genome, which turns on postsynaptic genes; and (3) another postsynaptic route starting from the newly expressed postsynaptic genes transferring information as a molecular cascade of biochemical consequences throughout the postsynaptic neuron.

It should now be clear that neurotransmission does not end when a neurotransmitter binds to a receptor or even when ion flows have been altered or second messengers have been created. Events such as these all start and end within milliseconds to seconds following release of presynaptic neurotransmitter. The ultimate goal of neurotransmission is to alter the biochemical activities of the postsynaptic target neuron in a profound and enduring manner. Since the postsynaptic DNA has to wait until molecular pony express messengers make their way from the postsynaptic receptors, often located on dendrites, to phosphoproteins within the neuron, or to transcription factors and genes in the postsynaptic neuron's cell nucleus, it can take a while for neurotransmission to begin influencing the postsynaptic target neuron's biochemical processes. The time it takes from receptor occupancy by neurotransmitter to gene expression is usually hours. Furthermore, since the last messenger triggered by neurotransmission – called a transcription factor – only initiates the very beginning of gene action, it takes even longer for the gene activation to be fully implemented via the series of biochemical events it triggers. These biochemical events can begin many hours to days after the neurotransmission occurred, and can last days or weeks once they are put in motion.

Thus, a brief puff of chemical neurotransmission from a presynaptic neuron can trigger a profound postsynaptic reaction that takes hours to days to develop and that can last days to weeks or even a lifetime. Every conceivable component of this entire process of chemical neurotransmission is a candidate for modification by drugs. Most psychotropic drugs act upon the processes that control chemical neurotransmission at the level of the neurotransmitters themselves or their enzymes and especially their receptors. Future psychotropic drugs will undoubtedly act directly upon the biochemical cascades, particularly upon those elements that control the expression of pre- and postsynaptic genes. Also, mental and neurological illnesses are known or suspected to affect these same aspects of chemical neurotransmission. The neuron is dynamically modifying its synaptic connections throughout its life, in response to learning, life experiences, genetic programming, epigenetic changes, drugs, and diseases, with chemical neurotransmission being the key aspect underlying the regulation of all these important processes.

https://doi.org/10.1017/9781108975292.002 Published online by Cambridge University Press