

Neurotransmitters and Their Receptors

Overview

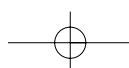
For the most part, neurons in the human brain communicate with one another by releasing chemical messengers called neurotransmitters. A large number of neurotransmitters are now known and more remain to be discovered. Neurotransmitters evoke postsynaptic electrical responses by binding to members of a diverse group of proteins called neurotransmitter receptors. There are two major classes of receptors: those in which the receptor molecule is also an ion channel, and those in which the receptor and ion channel are separate molecules. The former are called ionotropic receptors or ligand-gated ion channels, and give rise to fast postsynaptic responses that typically last only a few milliseconds. The latter are called metabotropic receptors, and they produce slower postsynaptic effects that may endure much longer. Abnormalities in the function of neurotransmitter systems contribute to a wide range of neurological and psychiatric disorders. As a result, many neuropharmacological therapies are based on drugs that affect neurotransmitter release, binding, and/or removal.

Categories of Neurotransmitters

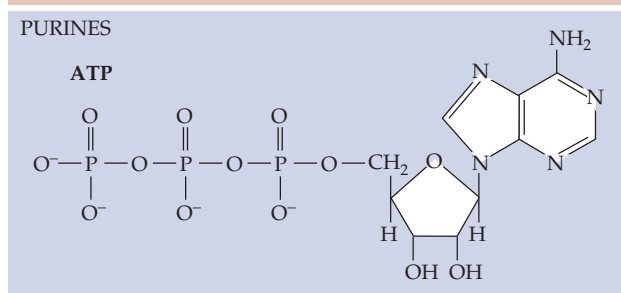
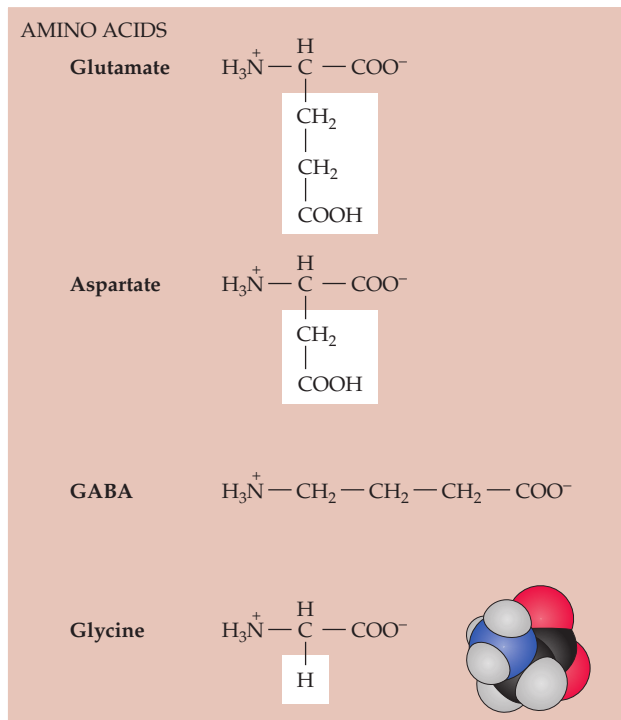
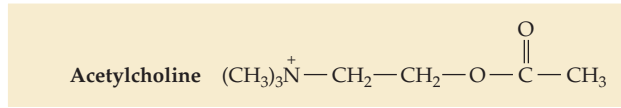
More than 100 different agents are known to serve as neurotransmitters. This large number of transmitters allows for tremendous diversity in chemical signaling between neurons. It is useful to separate this panoply of transmitters into two broad categories based simply on size (Figure 6.1). **Neuropeptides** are relatively large transmitter molecules composed of 3 to 36 amino acids. Individual amino acids, such as glutamate and GABA, as well as the transmitters acetylcholine, serotonin, and histamine, are much smaller than neuropeptides and have therefore come to be called **small-molecule neurotransmitters**. Within the category of small-molecule neurotransmitters, the **biogenic amines** (dopamine, norepinephrine, epinephrine, serotonin, and histamine) are often discussed separately because of their similar chemical properties and postsynaptic actions. The particulars of synthesis, packaging, release, and removal differ for each neurotransmitter (Table 6.1). This chapter will describe some of the main features of these transmitters and their postsynaptic receptors.

Acetylcholine

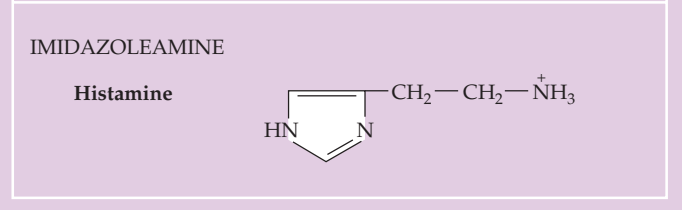
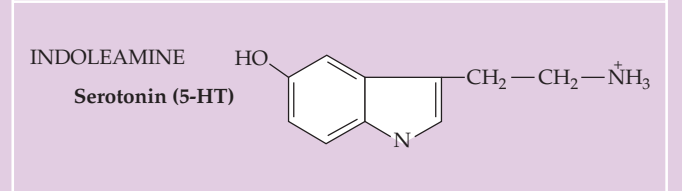
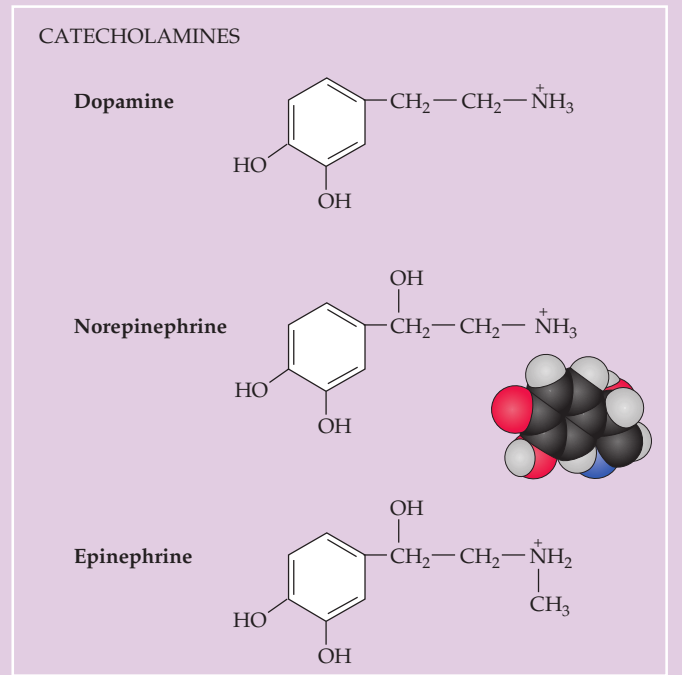
As mentioned in the previous chapter, acetylcholine (ACh) was the first substance identified as a neurotransmitter. In addition to the action of ACh as the neurotransmitter at skeletal neuromuscular junctions (see Chapter 5), as well as the neuromuscular synapse between the vagus nerve and cardiac



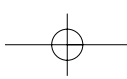
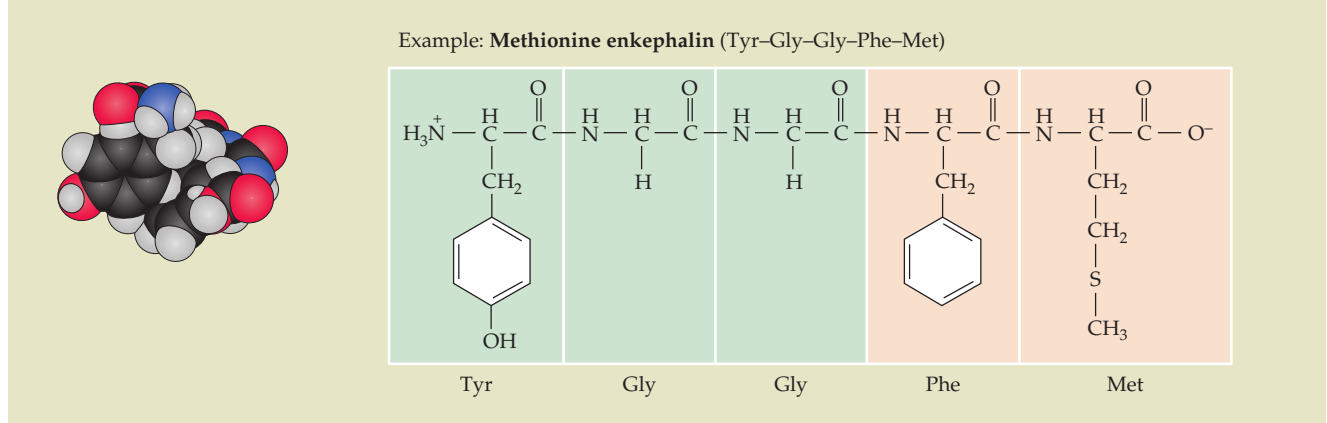
SMALL-MOLECULE NEUROTRANSMITTERS



BIOGENIC AMINES



PEPTIDE NEUROTRANSMITTERS (more than 100 peptides, usually 3–30 amino acids long)



◀ **Figure 6.1** Examples of small-molecule and peptide neurotransmitters. Small-molecule transmitters can be subdivided into acetylcholine, the amino acids, purines, and biogenic amines. The catecholamines, so named because they all share the catechol moiety (i.e., a hydroxylated benzene ring), make up a distinctive subgroup within the biogenic amines. Serotonin and histamine contain an indole ring and an imidazole ring, respectively. Size differences between the small-molecule neurotransmitters and the peptide neurotransmitters are indicated by the space-filling models for glycine, norepinephrine, and methionine enkephalin. (Carbon atoms are black, nitrogen atoms blue, and oxygen atoms red.)

muscle fibers, ACh serves as a transmitter at synapses in the ganglia of the visceral motor system, and at a variety of sites within the central nervous system. Whereas a great deal is known about the function of cholinergic transmission at neuromuscular junctions and ganglionic synapses, the actions of ACh in the central nervous system are not as well understood.

Acetylcholine is synthesized in nerve terminals from the precursors acetyl coenzyme A (acetyl CoA, which is synthesized from glucose) and choline, in a reaction catalyzed by choline acetyltransferase (CAT; Figure 6.2). Choline is present in plasma at a high concentration (about 10 mM) and is taken up into cholinergic neurons by a high-affinity Na⁺/choline transporter. After synthesis in the cytoplasm of the neuron, a vesicular ACh

TABLE 6.1
Functional Features of the Major Neurotransmitters

<i>Neurotransmitter</i>	<i>Postsynaptic effect^a</i>	<i>Precursor(s)</i>	<i>Rate-limiting step in synthesis</i>	<i>Removal mechanism</i>	<i>Type of vesicle</i>
ACh	Excitatory	Choline + acetyl CoA	CAT	AChE	Small, clear
Glutamate	Excitatory	Glutamine	Glutaminase	Transporters	Small, clear
GABA	Inhibitory	Glutamate	GAD	Transporters	Small, clear
Glycine	Inhibitory	Serine	Phosphoserine	Transporters	Small, clear
Catecholamines (epinephrine, norepinephrine, dopamine)	Excitatory	Tyrosine	Tyrosine hydroxylase	Transporters, MAO, COMT	Small dense-core, or large irregular dense-core
Serotonin (5-HT)	Excitatory	Tryptophan	Tryptophan hydroxylase	Transporters, MAO	Large, dense-core
Histamine	Excitatory	Histidine	Histidine decarboxylase	Transporters	Large, dense-core
ATP	Excitatory	ADP	Mitochondrial oxidative phosphorylation; glycolysis	Hydrolysis to AMP and adenosine	Small, clear
Neuropeptides	Excitatory and inhibitory	Amino acids (protein synthesis)	Synthesis and transport	Proteases	Large, dense-core
Endocannabinoids	Inhibits inhibition	Membrane lipids	Enzymatic modification of lipids	Hydrolysis by FAAH	None
Nitric oxide	Excitatory and inhibitory	Arginine	Nitric oxide synthase	Spontaneous oxidation	None

^aThe most common postsynaptic effect is indicated; the same transmitter can elicit postsynaptic excitation or inhibition depending on the nature of the ion channels affected by transmitter binding (see Chapter 7).

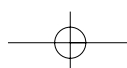
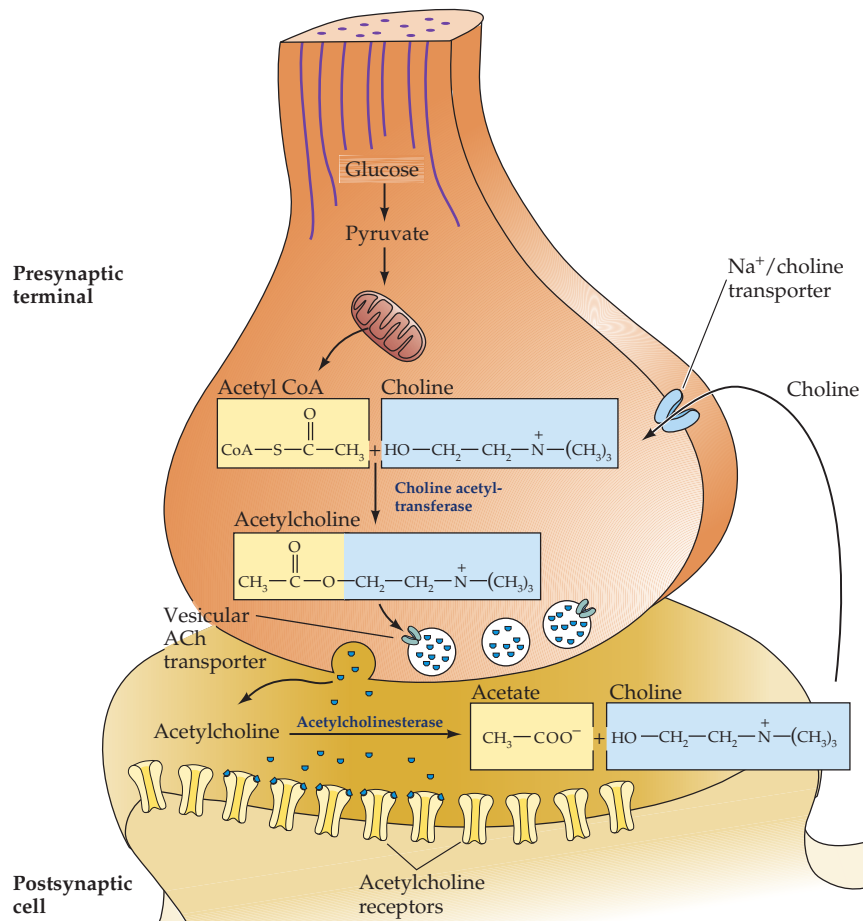


Figure 6.2 Acetylcholine metabolism in cholinergic nerve terminals. The synthesis of acetylcholine from choline and acetyl CoA requires choline acetyltransferase. Acetyl CoA is derived from pyruvate generated by glycolysis, while choline is transported into the terminals via a Na^+ -dependent transporter. Acetylcholine is loaded into synaptic vesicles via a vesicular transporter. After release, acetylcholine is rapidly metabolized by acetylcholinesterase, and choline is transported back into the terminal.

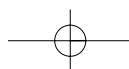


transporter loads approximately 10,000 molecules of ACh into each cholinergic vesicle.

In contrast to most other small-molecule neurotransmitters, the postsynaptic actions of ACh at many cholinergic synapses (the neuromuscular junction in particular) is not terminated by reuptake but by a powerful hydrolytic enzyme, acetylcholinesterase (AChE). This enzyme is concentrated in the synaptic cleft, ensuring a rapid decrease in ACh concentration after its release from the presynaptic terminal. AChE has a very high catalytic activity (about 5000 molecules of ACh per AChE molecule per second) and hydrolyzes ACh into acetate and choline. The choline produced by ACh hydrolysis is transported back into nerve terminals and used to resynthesize ACh.

Among the many interesting drugs that interact with cholinergic enzymes are the organophosphates. This group includes some potent chemical warfare agents. One such compound is the nerve gas "Sarin," which was made notorious after a group of terrorists released this gas in Tokyo's underground rail system. Organophosphates can be lethal because they inhibit AChE, causing ACh to accumulate at cholinergic synapses. This build-up of ACh depolarizes the postsynaptic cell and renders it refractory to subsequent ACh release, causing neuromuscular paralysis and other effects. The high sensitivity of insects to these AChE inhibitors has made organophosphates popular insecticides.

Many of the postsynaptic actions of ACh are mediated by the nicotinic ACh receptor (nAChR), so named because the CNS stimulant, nicotine, also



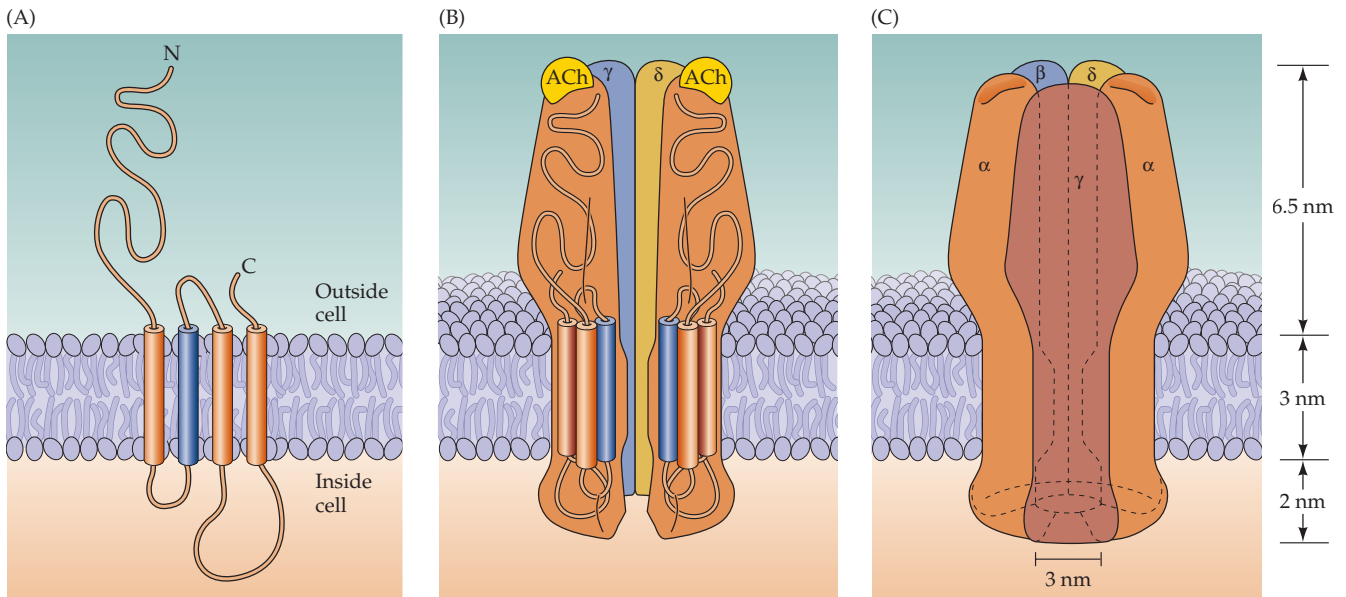
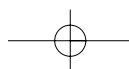


Figure 6.3 The structure of the nACh receptor/channel. (A) Each receptor subunit crosses the membrane four times. The membrane-spanning domain that lines the pore is shown in blue. (B) Five such subunits come together to form a complex structure containing 20 transmembrane domains that surround a central pore. (C) The openings at either end of the channel are very large—approximately 3 nm in diameter; even the narrowest region of the pore is approximately 0.6 nm in diameter. By comparison, the diameter of Na^+ or K^+ is less than 0.3 nm. (D) An electron micrograph of the nACh receptor, showing the actual position and size of the protein with respect to the membrane. (D from Toyoshima and Unwin, 1990.)

binds to these receptors. Nicotine consumption produces some degree of euphoria, relaxation, and eventually addiction (Box A), effects believed to be mediated in this case by nAChRs. Nicotinic receptors are the best-studied type of ionotropic neurotransmitter receptor. As described in Chapter 5, nAChRs are nonselective cation channels that generate excitatory postsynaptic responses. A number of biological toxins specifically bind to and block nicotinic receptors (Box B). The availability of these highly specific ligands—particularly a component of snake venom called α -bungarotoxin—has provided a valuable way to isolate and purify nAChRs. This pioneering work paved the way to cloning and sequencing the genes encoding the various subunits of the nAChR.

Based on these molecular studies, the nAChR is now known to be a large protein complex consisting of five subunits arranged around a central membrane-spanning pore (Figure 6.3). In the case of skeletal muscle AChRs, the receptor pentamer contains two α subunits, each of which binds one molecule of ACh. Because both ACh binding sites must be occupied for the channel to open, only relatively high concentrations of this neurotransmitter lead to channel activation. These subunits also bind other ligands, such as nicotine and α -bungarotoxin. At the neuromuscular junction, the two α subunits are combined with up to four other types of subunit— β , γ , δ , ϵ —in the ratio $2\alpha:\beta:\epsilon:\delta$. Neuronal nAChRs typically differ from those of muscle in that they lack sensitivity to α -bungarotoxin.



Box A

Addiction

Drug addiction is a chronic, relapsing disease with obvious medical, social, and political consequences. Addiction (also called substance dependence) is a persistent disorder of brain function in which compulsive drug use occurs despite serious negative consequences for the afflicted individual. The diagnostic manual of the American Psychiatric Association defines addiction in terms of both *physical* dependence and *psychological* dependence (in which an individual continues the drug-taking behavior despite obviously maladaptive consequences).

The range of substances that can generate this sort of dependence is wide; the primary agents of abuse at present are opioids, cocaine, amphetamines, marijuana, alcohol, and nicotine. Addiction to more “socially acceptable” agents such as alcohol and nicotine are sometimes regarded as less problematic, but in fact involve medical and behavioral consequences that are at least as great as for drugs of abuse that are considered more dangerous. Importantly, the phenomenon of addiction is

not limited to human behavior, but is demonstrable in laboratory animals. Most of these same agents are self-administered if primates, rodents, or other species are provided with the opportunity to do so.

In addition to a compulsion to take the agent of abuse, a major feature of addiction for many drugs is a constellation of negative physiological and emotional features, loosely referred to as “withdrawal syndrome,” that occur when the drug is not taken. The signs and symptoms of withdrawal are different for each agent of abuse, but in general are characterized by effects opposite those of the positive experience induced by the drug itself. Consider, as an example, cocaine, a drug that was estimated to be in regular use by 5 to 6 million Americans during the decade of the 1990s, with about 600,000 regular users either addicted or at high risk for addiction. The positive effects of the drug smoked or inhaled as a powder in the form of the alkaloidal free base is a “high” that is nearly immediate but generally lasts only a few minutes, typi-

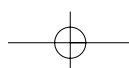
cally leading to a desire for additional drug in as little as 10 minutes to half an hour. The “high” is described as a feeling of well-being, self-confidence, and satisfaction. Conversely, when the drug is not available, frequent users experience depression, sleepiness, fatigue, drug-craving, and a general sense of malaise.

Another aspect of addiction to cocaine or other agents is tolerance, defined as a reduction in the response to the drug upon repeated administration. Tolerance occurs as a consequence of persistent use of a number of drugs but is particularly significant in drug addiction, since it progressively increases the dose needed to experience the desired effects.

Although it is fair to say that the neurobiology of addiction is incompletely understood, for cocaine and many other agents of abuse the addictive effects involve activation of dopamine receptors in critical brain regions involved in motivation and emotional reinforcement (see Chapter 28). The most important of these areas is the midbrain dopamine system,

toxin, and comprise only two receptor subunit types (α and β), which are present in a ratio of $3\alpha:2\beta$. In all cases, however, five individual subunits assemble to form a functional, cation-selective nACh receptor.

Each subunit of the nAChR molecule contains four transmembrane domains that make up the ion channel portion of the receptor, and a long extracellular region that makes up the ACh-binding domain (Figure 6.3A). Unraveling the molecular structure of this region of the nACh receptor has provided insight into the mechanisms that allow ligand-gated ion channels to respond rapidly to neurotransmitters: The intimate association of the ACh binding sites with the pore of the channel presumably accounts for the rapid response to ACh (Figure 6.3B–D). Indeed, this general arrangement is characteristic of *all* of the ligand-gated ion channels at fast-acting synapses, as summarized in Figure 6.4. Thus, the nicotinic receptor has served as a paradigm for studies of other ligand-gated ion channels, at the same time leading to a much deeper appreciation of several neuromuscular diseases (Box C).



especially its projections from the ventral-tegmental area to the nucleus accumbens. Agents such as cocaine appear to act by raising dopamine levels in these areas, making this transmitter more available to receptors by interfering with re-uptake of synaptically released dopamine by the dopamine transporter. The reinforcement and motivation of drug-taking behaviors is thought to be related to the projections to the nucleus accumbens.

The most common opioid drug of abuse is heroin. Heroin is a derivative of the opium poppy and is not legally available for clinical purposes in the United States. The number of heroin addicts in the United States is estimated to be between 750,000 and a million individuals. The positive feelings produced by heroin, generally described as the “rush,” are often compared to the feeling of sexual orgasm and begin in less than a minute after intravenous injection. There is then a feeling of general well-being (referred to as “on the nod”) that lasts about an hour. The symptoms of withdrawal can be

intense; these are restlessness, irritability, nausea, muscle pain, depression, sleeplessness, and a sense of anxiety and malaise. The reinforcing aspects of the drug entail the same dopaminergic circuitry in the ventral tegmental area and nucleus accumbens as does cocaine, although additional areas are certainly involved, particularly the sites of opioid receptors described in Chapter 9.

Interestingly, addiction to heroin or any other agent is not an inevitable consequence of drug use, but depends critically on the environment. For instance, returning veterans who were heroin addicts in Vietnam typically lost their addiction upon returning to the United States. Likewise, patients given other opioids (e.g., morphine) for painful conditions rarely become addicts.

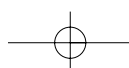
The treatment of any form of addiction is difficult and must be tailored to the circumstances of the individual. In addition to treating acute problems of withdrawal and “detoxification,” patterns of behavior must be changed that may take months or years. Addiction is thus a chronic disease state that requires

continual monitoring during the lifetime of susceptible individuals.

References

- AMERICAN PSYCHIATRIC ASSOCIATION (1994) *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edition (DSM IV). Washington, D.C.
- HYMAN, S. E. AND R. C. MALENKA (2001) Addiction and the brain: The neurobiology of compulsion and its persistence. *Nature Rev. Neurosci.* 2: 695–703.
- LAAKSO, A., A. R. MOHN, R. R. GAINETDINOV AND M. G. CARON (2002) Experimental genetic approaches to addiction. *Neuron* 36: 213–228.
- O'BRIEN, C. P. (2001) Goodman and Gilman's *The Pharmaceutical Basis of Therapeutics*, 10th Edition. New York: McGraw-Hill, Chapter 24, pp. 621–642..

A second class of ACh receptors is activated by muscarine, a poisonous alkaloid found in some mushrooms (see Box B), and thus they are referred to as muscarinic ACh receptors (mAChRs). mAChRs are metabotropic and mediate most of the effects of ACh in brain. Several subtypes of mAChR are known (Figure 6.5). Muscarinic ACh receptors are highly expressed in the striatum and various other forebrain regions, where they exert an inhibitory influence on dopamine-mediated motor effects. These receptors are also found in the ganglia of the peripheral nervous system. Finally, they mediate peripheral cholinergic responses of autonomic effector organs—such as heart, smooth muscle, and exocrine glands—and are responsible for the inhibition of heart rate by the vagus nerve. Numerous drugs act as mACh receptor agonists or antagonists, but most of these do not discriminate between different types of muscarinic receptors and often produce side effects. Nevertheless, mACh blockers that are therapeutically useful include atropine (used to dilate the pupil), scopolamine (effective in preventing motion sickness), and ipratropium (useful in the treatment of asthma).



Box B

Neurotoxins that Act on Postsynaptic Receptors

Poisonous plants and venomous animals are widespread in nature. The toxins they produce have been used for a variety of purposes, including hunting, healing, mind-altering, and, more recently, research. Many of these toxins have potent actions on the nervous system, often interfering with synaptic transmission by targeting neurotransmitter receptors. The poisons found in some organisms contain a single type of toxin, whereas others contain a mixture of tens or even hundreds of toxins.

Given the central role of ACh receptors in mediating muscle contraction at neuromuscular junctions in numerous species, it is not surprising that a large number of natural toxins interfere with transmission at this synapse. In fact, the classification of nicotinic and muscarinic ACh receptors is based on the sensitivity of these receptors to the toxic plant alkaloids nicotine and muscarine, which activate nicotinic and muscarinic ACh receptors, respectively. Nicotine is derived

from the dried leaves of the tobacco plant *Nicotinia tabacum*, and muscarine is from the poisonous red mushroom *Amanita muscaria*. Both toxins are stimulants that produce nausea, vomiting, mental confusion, and convulsions. Muscarine poisoning can also lead to circulatory collapse, coma, and death.

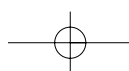
The poison α -bungarotoxin, one of many peptides that together make up the venom of the banded krait, *Bungarus multicinctus* (Figure A), blocks transmission at neuromuscular junctions and is used by the snake to paralyze its prey. This 74-amino-acid toxin blocks neuromuscular transmission by irreversibly binding to nicotinic ACh receptors, thus preventing ACh from opening postsynaptic ion channels. Paralysis ensues because skeletal muscles can no longer be activated by motor neurons. As a result of its specificity and its high affinity for nicotinic ACh receptors, α -bungarotoxin has contributed greatly to understanding the ACh receptor mole-

cule. Other snake toxins that block nicotinic ACh receptors are cobra α -neurotoxin and the sea snake peptide erabutoxin. The same strategy used by these snakes to paralyze prey was adopted by South American Indians who used curare, a mixture of plant toxins from *Chondodendron tomentosum*, as an arrowhead poison to immobilize their quarry. Curare also blocks nicotinic ACh receptors; the active agent is the alkaloid δ -tubocurarine.

Another interesting class of animal toxins that selectively block nicotinic ACh and other receptors includes the peptides produced by fish-hunting marine cone snails (Figure B). These colorful snails kill small fish by “shooting” venomous darts into them. The venom contains hundreds of peptides, known as the conotoxins, many of which target proteins that are important in synaptic transmission. There are conotoxin peptides that block Ca^{2+} channels, Na^{+} channels, glutamate receptors, and ACh



(A) The banded krait *Bungarus multicinctus*. (B) A marine cone snail (*Conus* sp.) uses venomous darts to kill a small fish. (C) Betel nuts, *Areca catechu*, growing in Malaysia. (A, Robert Zappalorti/Photo Researchers, Inc.; B, Zoya Maslak and Baldomera Olivera, University of Utah; C, Fletcher Baylis/Photo Researchers, Inc.)



receptors. The array of physiological responses produced by these peptides all serve to immobilize any prey unfortunate enough to encounter the cone snail. Many other organisms, including other mollusks, corals, worms and frogs, also utilize toxins containing specific blockers of ACh receptors.

Other natural toxins have mind- or behavior-altering effects and in some cases have been used for thousands of years by shamans and, more recently, physicians. Two examples are plant alkaloid toxins that block muscarinic ACh receptors: atropine from deadly nightshade (belladonna), and scopolamine from henbane. Because these plants grow wild in many parts of the world, exposure is not unusual, and poisoning by either toxin can also be fatal.

Another postsynaptic neurotoxin that, like nicotine, is used as a social drug is found in the seeds from the betel nut, *Areca catechu* (Figure C). Betel nut chewing, although unknown in the United States, is practiced by up to 25% of the population in India, Bangladesh, Ceylon, Malaysia, and the Philippines. Chewing these nuts produces a euphoria caused by arecoline, an alkaloid agonist of nicotinic ACh receptors. Like nicotine, arecoline is an addictive central nervous system stimulant.

Many other neurotoxins alter transmission at noncholinergic synapses. For example, amino acids found in certain

mushrooms, algae, and seeds are potent glutamate receptor agonists. The excitotoxic amino acids kainate, from the red alga *Digenea simplex*, and quisqualate, from the seed of *Quisqualis indica*, are used to distinguish two families of non-NMDA glutamate receptors (see text). Other neurotoxic amino acid activators of glutamate receptors include ibotenic acid and acromelic acid, both found in mushrooms, and domoate, which occurs in algae, seaweed, and mussels. Another large group of peptide neurotoxins blocks glutamate receptors. These include the α -agatoxins from the funnel web spider, NSTX-3 from the orb weaver spider, jorotoxin from the Joro spider, and β -philanthotoxin from wasp venom, as well as many cone snail toxins.

All the toxins discussed so far target excitatory synapses. The inhibitory GABA and glycine receptors, however, have not been overlooked by the exigencies of survival. Strychnine, an alkaloid extracted from the seeds of *Strychnos nux-vomica*, is the only drug known to have specific actions on transmission at glycinergic synapses. Because the toxin blocks glycine receptors, strychnine poisoning causes overactivity in the spinal cord and brainstem, leading to seizures. Strychnine has long been used commercially as a poison for rodents, although alternatives such as the anticoagulant coumadin are now more popular because they are safer for humans. Neu-

rotoxins that block GABA_A receptors include plant alkaloids such as bicuculline from Dutchman's breeches and picrotoxin from *Anamerta cocculus*. Dieldrin, a commercial insecticide, also blocks these receptors. These agents are, like strychnine, powerful central nervous system stimulants. Muscimol, a mushroom toxin that is a powerful depressant as well as a hallucinogen, activates GABA_A receptors. A synthetic analogue of GABA, baclofen, is a GABA_B agonist that reduces EPSPs in some brainstem neurons and is used clinically to reduce the frequency and severity of muscle spasms.

Chemical warfare between species has thus given rise to a staggering array of molecules that target synapses throughout the nervous system. Although these toxins are designed to defeat normal synaptic transmission, they have also provided a set of powerful tools to understand postsynaptic mechanisms.

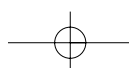
References

- ADAMS, M. E. AND B. M. OLIVERA (1994) Neurotoxins: Overview of an emerging research technology. *TINS* 17: 151–155.
- HUCHO, F. AND Y. OVCHINNIKOV (1990) *Toxins as Tools in Neurochemistry*. Berlin: Walter de Gruyter.
- MYERS, R. A., L. J. CRUZ, J. E. RIVIER AND B. M. OLIVERA (1993) *Conus* peptides as chemical probes for receptors and ion channels. *Chem. Rev.* 93: 1923–1926.

Glutamate

Glutamate is the most important transmitter in normal brain function. Nearly all excitatory neurons in the central nervous system are glutamatergic, and it is estimated that over half of all brain synapses release this agent. Glutamate plays an especially important role in clinical neurology because elevated concentrations of extracellular glutamate, released as a result of neural injury, are toxic to neurons (Box D).

Glutamate is a nonessential amino acid that does not cross the blood-brain barrier and therefore must be synthesized in neurons from local precursors. The most prevalent precursor for glutamate synthesis is glutamine, which is released by glial cells. Once released, glutamine is taken up into presynaptic



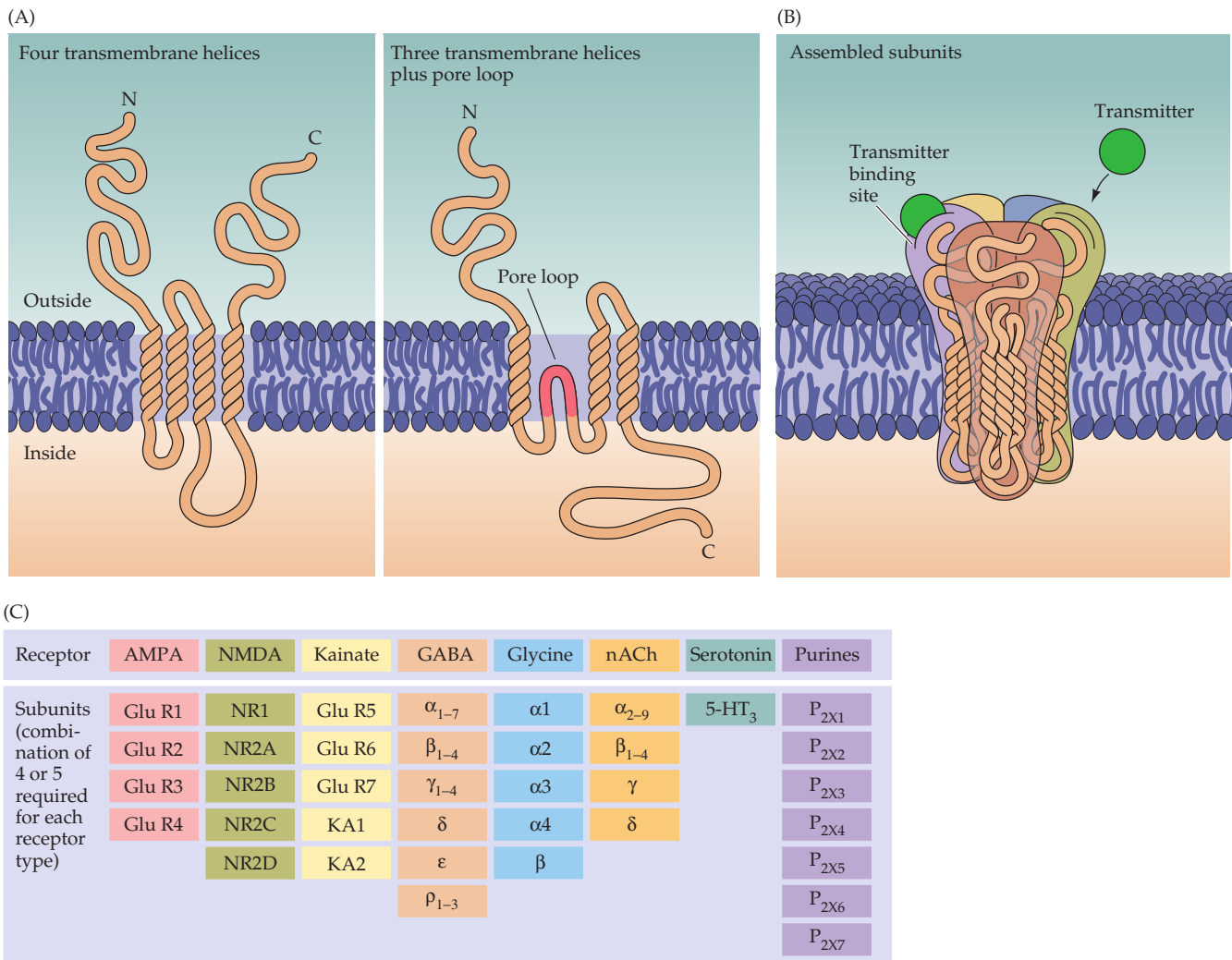
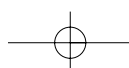


Figure 6.4 The general architecture of ligand-gated receptors. (A) One of the subunits of a complete receptor. The long N-terminal region forms the ligand-binding site, while the remainder of the protein spans the membrane either four times (left) or three times (right). (B) Assembly of either four or five subunits into a complete receptor. (C) A diversity of subunits come together to form functional ionotropic neurotransmitter receptors.

terminals and metabolized to glutamate by the mitochondrial enzyme glutaminase (Figure 6.6). Glutamate can also be synthesized by transamination of 2-oxoglutarate, an intermediate of the tricarboxylic acid cycle. Hence, some of the glucose metabolized by neurons can also be used for glutamate synthesis.

The glutamate synthesized in the presynaptic cytoplasm is packaged into synaptic vesicles by transporters, termed VGLUT. At least three different VGLUT genes have been identified. Once released, glutamate is removed from the synaptic cleft by the excitatory amino acid transporters (EAATs). There are five different types of high-affinity glutamate transporters exist, some of which are present in glial cells and others in presynaptic terminals. Glutamate taken up by glial cells is converted into glutamine by the enzyme



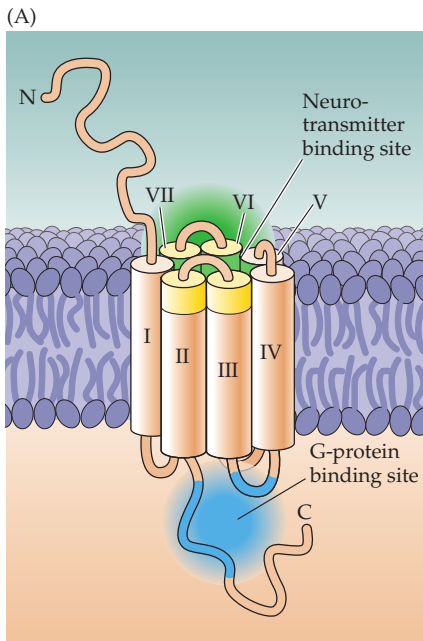


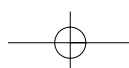
Figure 6.5 Structure and function of metabotropic receptors. (A) The transmembrane architecture of metabotropic receptors. These monomeric proteins contain seven transmembrane domains. Portions of domains II, III, VI, and VII make up the neurotransmitter-binding region. G-proteins bind to both the loop between domains V and VI and to portions of the C-terminal region. (B) Varieties of metabotropic neurotransmitter receptors.

(B)

Receptor class	Glutamate	GABA _B	Dopamine	NE, Epi	Histamine	Serotonin	Purines	Muscarinic
Receptor subtype	Class I	GABA _B R1	D1 _A	α1	H1	5-HT 1	A type	M1
	mGlu R1	GABA _B R2	D1 _B	α2	H2	5-HT 2	A1	M2
	mGlu R5		D2	β1	H3	5-HT 3	A2a	M3
	Class II		D3	β2		5-HT 4	A2b	M4
	mGlu R2		D4	β3		5-HT 5	A3	M5
	mGlu R3					5-HT 6	P type	
	Class III					5-HT 7	P2x	
	mGlu R4						P2y	
	mGlu R6						P2z	
	mGlu R7						P2t	
	mGlu R8						P2u	

glutamine synthetase; glutamine is then transported out of the glial cells and into nerve terminals. In this way, synaptic terminals cooperate with glial cells to maintain an adequate supply of the neurotransmitter. This overall sequence of events is referred to as the **glutamate-glutamine cycle** (see Figure 6.6).

Several types of glutamate receptors have been identified. Three of these are ionotropic receptors called, respectively, **NMDA receptors, AMPA receptors, and kainate receptors** (Figure 6.4C). These glutamate receptors are named after the agonists that activate them: NMDA (*N*-methyl-*D*-aspartate), AMPA (α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate), and kainic acid. All of the ionotropic glutamate receptors are nonselective cation channels similar to the nAChR, allowing the passage of Na⁺ and K⁺, and in some cases small amounts of Ca²⁺. Hence AMPA, kainate, and NMDA receptor activation always produces excitatory postsynaptic responses. Like other ionotropic receptors, AMPA/kainate and NMDA receptors are also formed



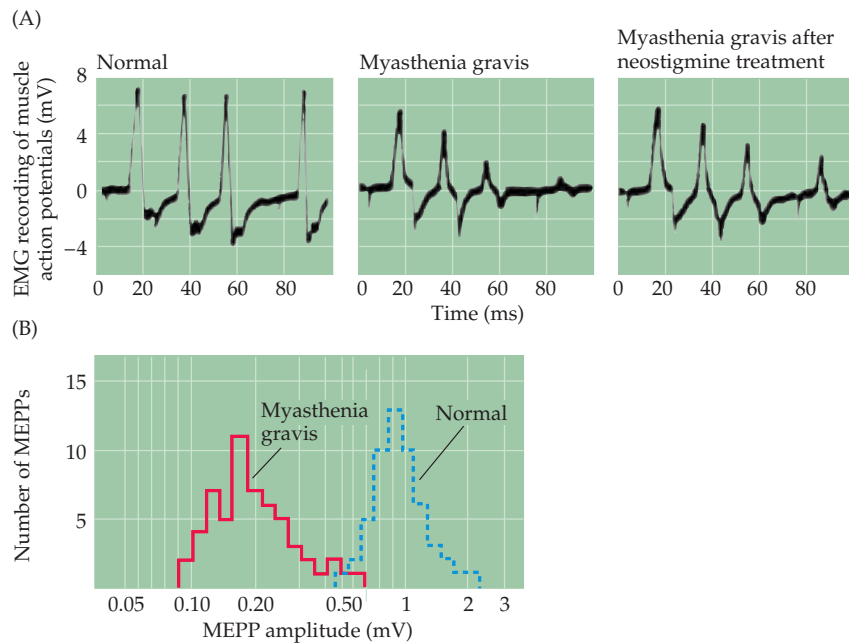
Box C

Myasthenia Gravis: An Autoimmune Disease of Neuromuscular Synapses

Myasthenia gravis is a disease that interferes with transmission between motor neurons and skeletal muscle fibers and afflicts approximately 1 of every 200,000 people. Originally described by the British physician Thomas Willis in 1685, the hallmark of the disorder is muscle weakness, particularly during sustained activity. Although the course is variable, myasthenia commonly affects muscles controlling the eyelids (resulting in drooping of the eyelids, or ptosis) and eye movements (resulting in double vision, or diplopia). Muscles controlling facial expression, chewing, swallowing, and speaking are other common targets.

An important indication of the cause of myasthenia gravis came from the clinical observation that the muscle weakness improves following treatment with inhibitors of acetylcholinesterase, the enzyme that normally degrades acetylcholine at the neuromuscular junction. Studies of muscle obtained by biopsy from myasthenic patients showed that both end plate potentials (EPPs) and miniature end plate potentials (MEPPs) are much smaller than normal (see figure; also see Chapter 5). Because both the frequency of MEPPs and the quantal content of EPPs are normal, it seemed likely that myasthenia gravis entails a disorder of the postsynaptic muscle cells. Indeed, electron microscopy shows that the structure of neuromuscular junctions is altered, obvious changes being a widening of the synaptic cleft and an apparent reduction in the number of acetylcholine receptors in the postsynaptic membrane.

A chance observation in the early 1970s led to the discovery of the underlying cause of these changes. Jim Patrick and Jon Lindstrom, then working at the Salk Institute, were attempting to raise antibodies to nicotinic acetylcholine receptors by immunizing rabbits with



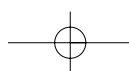
(A) Myasthenia gravis reduces the efficiency of neuromuscular transmission. Electromyographs show muscle responses elicited by stimulating motor nerves. In normal individuals, each stimulus in a train evokes the same contractile response. In contrast, transmission rapidly fatigues in myasthenic patients, although it can be partially restored by administration of acetylcholinesterase inhibitors such as neostigmine. (B) Distribution of MEPP amplitudes in muscle fibers from myasthenic patients (solid line) and controls (dashed line). The smaller size of MEPPs in myasthenics is due to a diminished number of postsynaptic receptors. (A after Harvey et al., 1941; B after Elmquist et al., 1964.)

the receptors. Unexpectedly, the immunized rabbits developed muscle weakness that improved after treatment with acetylcholinesterase inhibitors. Subsequent work showed that the blood of myasthenic patients contains antibodies directed against the acetylcholine receptor, and that these antibodies are present at neuromuscular synapses. Removal of antibodies by plasma exchange improves the weakness. Finally, injecting the serum of myasthenic patients into mice produces myasthenic effects (because the serum carries circulating antibodies).

These findings indicate that myasthenia gravis is an autoimmune disease that targets nicotinic acetylcholine receptors. The immune response

reduces the number of functional receptors at the neuromuscular junction and can eventually destroy them altogether, diminishing the efficiency of synaptic transmission; muscle weakness thus occurs because motor neurons are less capable of exciting the postsynaptic muscle cells. This causal sequence also explains why cholinesterase inhibitors alleviate the signs and symptoms of myasthenia: The inhibitors increase the concentration of acetylcholine in the synaptic cleft, allowing more effective activation of those postsynaptic receptors not yet destroyed by the immune system.

Despite all these insights, it is still not clear what triggers the immune system to produce an autoimmune



response to acetylcholine receptors. Surgical removal of the thymus is beneficial in young patients with hyperplasia of the thymus, though precisely how the thymus contributes to myasthenia gravis is incompletely understood. Many patients are treated with combi-

nations of immunosuppression and cholinesterase inhibitors.

References

ELMQVIST, D., W. W. HOFMANN, J. KUGELBERG AND D. M. J. QUASTEL (1964) An electrophysiological investigation of neuromuscular

transmission in myasthenia gravis. *J. Physiol. (Lond.)* 174: 417–434.

PATRICK, J. AND J. LINDSTROM (1973) Autoimmune response to acetylcholine receptor. *Science* 180: 871–872.

VINCENT, A. (2002) Unravelling the pathogenesis of myasthenia gravis. *Nature Rev. Immunol.* 2: 797–804.

from the association of several protein subunits that can combine in many ways to produce a large number of receptor isoforms (see Figure 6.4C).

NMDA receptors have especially interesting properties (Figure 6.7A). Perhaps most significant is the fact that NMDA receptor ion channels allow the entry of Ca^{2+} in addition to monovalent cations such as Na^+ and K^+ . As a result, EPSPs produced by NMDA receptors can increase the concentration of Ca^{2+} within the postsynaptic neuron; the Ca^{2+} concentration change can then act as a second messenger to activate intracellular signaling cascades (see Chapter 7). Another key property is that they bind extracellular Mg^{2+} . At hyperpolarized membrane potentials, this ion blocks the pore of the NMDA receptor channel. Depolarization, however, pushes Mg^{2+} out of the pore, allowing other cations to flow. This property provides the basis for a voltage-dependence to current flow through the receptor (dashed line in Figure 6.7B) and means that NMDA receptors pass cations (most notably Ca^{2+})

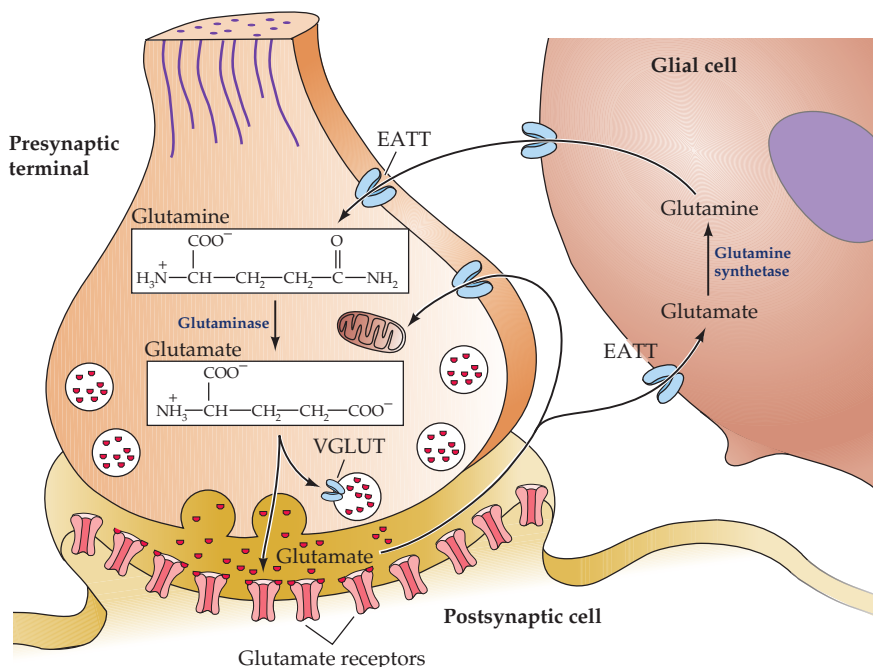
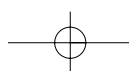


Figure 6.6 Glutamate synthesis and cycling between neurons and glia. The action of glutamate released into the synaptic cleft is terminated by uptake into neurons and surrounding glial cells via specific transporters. Within the nerve terminal, the glutamine released by glial cells and taken up by neurons is converted back to glutamate. Glutamate is transported into cells via excitatory amino acid transporters (EATs) and loaded into synaptic vesicles via vesicular glutamate transporters (VGLUT).



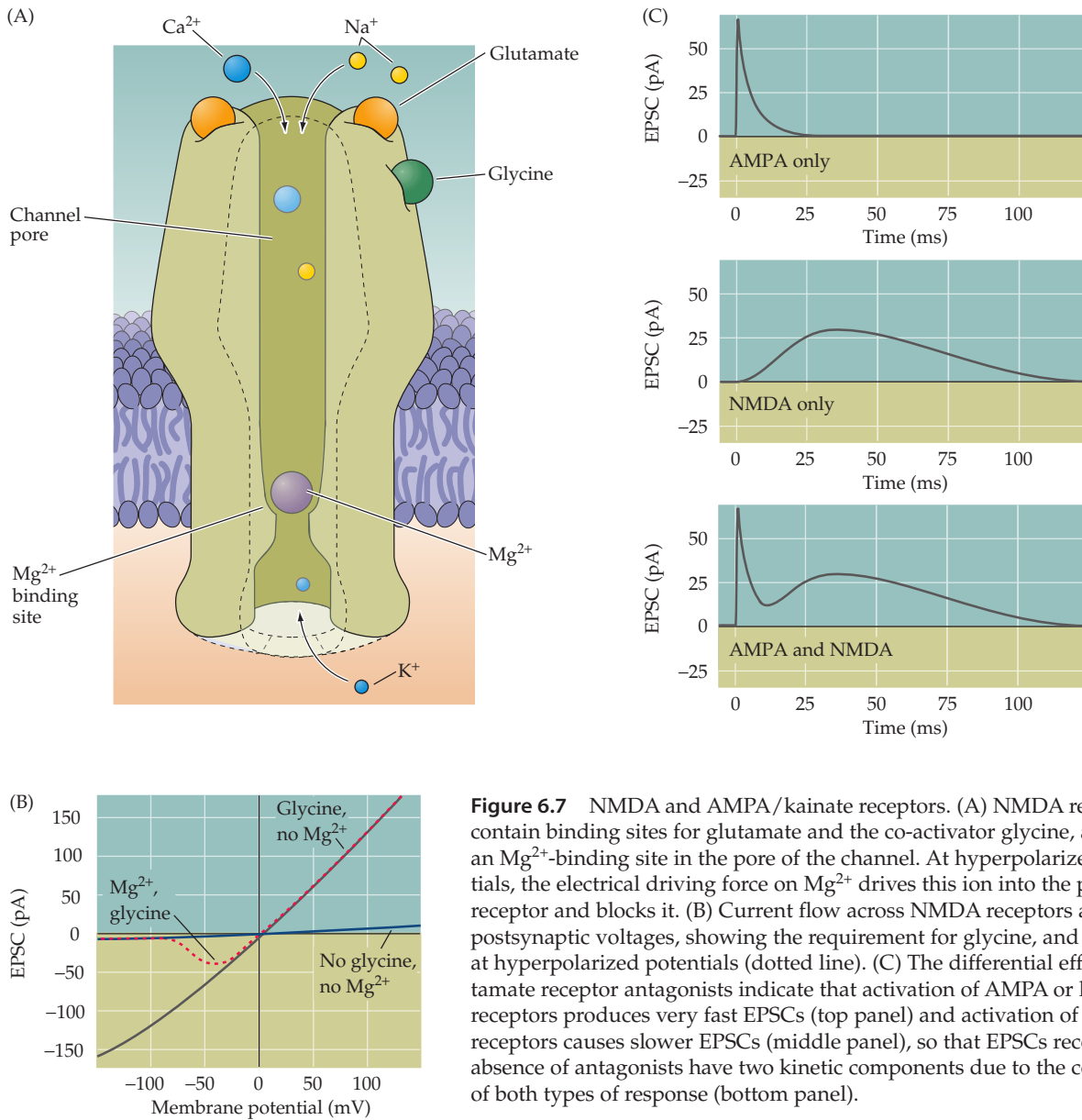
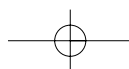


Figure 6.7 NMDA and AMPA/kainate receptors. (A) NMDA receptors contain binding sites for glutamate and the co-activator glycine, as well as an Mg^{2+} -binding site in the pore of the channel. At hyperpolarized potentials, the electrical driving force on Mg^{2+} drives this ion into the pore of the receptor and blocks it. (B) Current flow across NMDA receptors at a range of postsynaptic voltages, showing the requirement for glycine, and Mg^{2+} block at hyperpolarized potentials (dotted line). (C) The differential effects of glutamate receptor antagonists indicate that activation of AMPA or kainate receptors produces very fast EPSCs (top panel) and activation of NMDA receptors causes slower EPSCs (middle panel), so that EPSCs recorded in the absence of antagonists have two kinetic components due to the contribution of both types of response (bottom panel).

only during depolarization of the postsynaptic cell, due to either activation of a large number of excitatory inputs and/or by repetitive firing of action potentials in the presynaptic cell. These properties are widely thought to be the basis for some forms of information storage at synapses, such as memory, as described in Chapter 24. Another unusual property of NMDA receptors is that opening the channel of this receptor requires the presence of a co-agonist, the amino acid glycine (Figure 6.7A,B). There are at least five forms of NMDA receptor subunits (NMDA-R1, and NMDA-R2A through NMDA-R2D); different synapses have distinct combinations of these subunits, producing a variety of NMDA receptor-mediated postsynaptic responses.

Whereas some glutamatergic synapses have only AMPA or NMDA receptors, most possess both AMPA and NMDA receptors. An antagonist of



NMDA receptors, APV (2-amino-5-phosphono-valerate), is often used to differentiate between the two receptor types. The use of this drug has also revealed differences between the EPSPs produced by NMDA and those produced by AMPA/kainate receptors, such as the fact that the synaptic currents produced by NMDA receptors are slower and longer-lasting than the those produced by AMPA/kainate receptors (see Figure 6.7C).

In addition to these ionotropic glutamate receptors, there are three types of metabotropic glutamate receptor (mGluRs) (Figure 6.5). These receptors, which modulate postsynaptic ion channels indirectly, differ in their coupling to intracellular signal transduction pathways (see Chapter 7) and in their sensitivity to pharmacological agents. Activation of many of these receptors leads to inhibition of postsynaptic Ca^{2+} and Na^+ channels. Unlike the excitatory ionotropic glutamate receptors, mGluRs cause slower postsynaptic responses that can either increase or decrease the excitability of postsynaptic cells. As a result the physiological roles of mGluRs are quite varied.

GABA and Glycine

Most inhibitory synapses in the brain and spinal cord use either γ -aminobutyric acid (GABA) or glycine as neurotransmitters. Like glutamate, GABA was identified in brain tissue during the 1950s. The details of its synthesis and degradation were worked out shortly thereafter by the work of Ernst Florey and Eugene Roberts. During this era, David Curtis and Jeffrey Watkins first showed that GABA can inhibit action potential firing in mammalian neurons. Subsequent studies by Edward Kravitz and colleagues established that GABA serves as an inhibitory transmitter at lobster neuromuscular synapses. It is now known that as many as a third of the synapses in the brain use GABA as their inhibitory neurotransmitter. GABA is most commonly found in local circuit interneurons, although cerebellar Purkinje cells provide an example of a GABAergic projection neuron (see Chapter 18).

The predominant precursor for GABA synthesis is glucose, which is metabolized to glutamate by the tricarboxylic acid cycle enzymes (pyruvate and glutamine can also act as precursors). The enzyme glutamic acid decarboxylase (GAD), which is found almost exclusively in GABAergic neurons, catalyzes the conversion of glutamate to GABA (Figure 6.8A). GAD requires a cofactor, pyridoxal phosphate, for activity. Because pyridoxal phosphate is derived from vitamin B_6 , a B_6 deficiency can lead to diminished GABA synthesis. The significance of this became clear after a disastrous series of infant deaths was linked to the omission of vitamin B_6 from infant formula. This lack of B_6 resulted in a large reduction in the GABA content of the brain, and the subsequent loss of synaptic inhibition caused seizures that in some cases were fatal. Once GABA is synthesized, it is transported into synaptic vesicles via a vesicular inhibitory amino acid transporter (VIATT).

The mechanism of GABA removal is similar to that for glutamate: Both neurons and glia contain high-affinity transporters for GABA, termed GATs (several forms of GAT have been identified). Most GABA is eventually converted to succinate, which is metabolized further in the tricarboxylic acid cycle that mediates cellular ATP synthesis. The enzymes required for this degradation, GABA transaminase and succinic semialdehyde dehydrogenase, are mitochondrial enzymes. Inhibition of GABA breakdown causes a rise in tissue GABA content and an increase in the activity of inhibitory neurons. There are also other pathways for degradation of GABA. The most noteworthy of these results in the production of γ -hydroxybutyrate, a GABA derivative that has been abused as a "date rape" drug. Oral adminis-

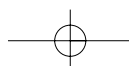
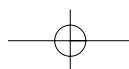
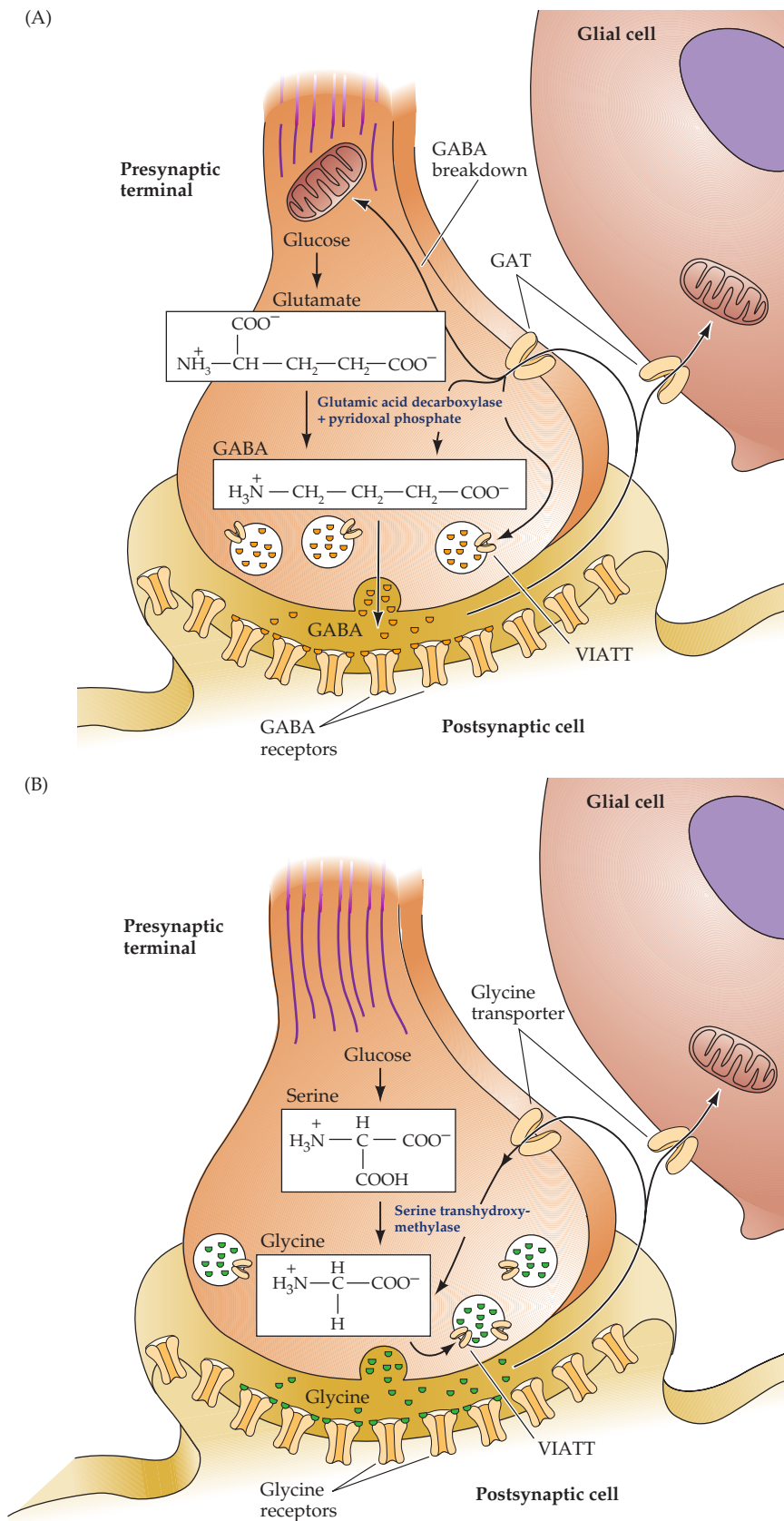


Figure 6.8 Synthesis, release, and reuptake of the inhibitory neurotransmitters GABA and glycine. (A) GABA is synthesized from glutamate by the enzyme glutamic acid decarboxylase, which requires pyridoxal phosphate. (B) Glycine can be synthesized by a number of metabolic pathways; in the brain, the major precursor is serine. High-affinity transporters terminate the actions of these transmitters and return GABA or glycine to the synaptic terminals for reuse, with both transmitters being loaded into synaptic vesicles via the vesicular inhibitory amino acid transporter (VIATT).



Box D

Excitotoxicity Following Acute Brain Injury

Excitotoxicity refers to the ability of glutamate and related compounds to destroy neurons by prolonged excitatory synaptic transmission. Normally, the concentration of glutamate released into the synaptic cleft rises to high levels (approximately 1 mM), but it remains at this concentration for only a few milliseconds. If abnormally high levels of glutamate accumulate in the cleft, the excessive activation of neuronal glutamate receptors can literally excite neurons to death.

The phenomenon of excitotoxicity was discovered in 1957 when D. R. Lucas and J. P. Newhouse serendipitously found that feeding sodium glutamate to infant mice destroys neurons in the retina. Roughly a decade later, John Olney at Washington University extended this discovery by showing that regions of glutamate-induced neuronal loss can occur throughout the brain. The damage was evidently restricted to the postsynaptic cells—the dendrites of the target neurons were grossly swollen—while the presynaptic terminals were spared. Olney also examined the relative potency of glutamate analogs and found that their neurotoxic actions paralleled their ability to activate postsynaptic glutamate receptors. Furthermore, glutamate receptor antagonists were effective in blocking the neurotoxic effects of glutamate. In light of this evidence, Olney postulated that glutamate destroys neurons by a mechanism similar to transmission at excitatory glutamatergic syn-

apses, and coined the term *excitotoxic* to refer to this pathological effect.

Evidence that excitotoxicity is an important cause of neuronal damage after brain injury has come primarily from studying the consequences of reduced blood flow. The most common cause of reduced blood flow to the brain (ischemia) is the occlusion of a cerebral blood vessel (i.e., a stroke; see Appendix 3). The idea that excessive synaptic activity contributes to ischemic injury emerged from the observation that concentrations of glutamate and aspartate in the extracellular space around neurons increase during ischemia. Moreover, microinjection of glutamate receptor antagonists in experimental animals protects neurons from ischemia-induced damage. Together, these findings imply that extracellular accumulation of glutamate during ischemia activates glutamate receptors excessively, and that this somehow triggers a chain of events that leads to neuronal death. The reduced supply of oxygen and glucose presumably elevates extracellular glutamate levels by slowing the energy-dependent removal of glutamate at synapses.

Excitotoxic mechanisms have now been shown to be involved in other acute forms of neuronal insult, including hypoglycemia, trauma, and repeated intense seizures (called status epilepticus). Understanding excitotoxicity therefore has important implications for treating a variety of neurological disorders. For instance, a blockade of glutamate

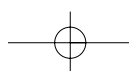
receptors could, in principle, protect neurons from injury due to stroke, trauma, or other causes. Unfortunately, clinical trials of glutamate receptor antagonists have not led to much improvement in the outcome of stroke. The ineffectiveness of this quite logical treatment is probably due to several factors, one of which is that substantial excitotoxic injury occurs quite soon after ischemia, prior to the typical initiation of treatment. It is also likely that excitotoxicity is only one of several mechanisms by which ischemia damages neurons, other candidates including damage secondary to inflammation. Pharmacological interventions that target all these mechanisms nonetheless hold considerable promise for minimizing brain injury after stroke and other causes.

References

- LUCAS, D. R. AND J. P. NEWHOUSE (1957) The toxic effects of sodium L-glutamate on the inner layers of the retina. *Arch. Ophthalmol.* 58: 193–201.
- OLNEY, J. W. (1969) Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. *Science* 164: 719–721.
- OLNEY, J. W. (1971) Glutamate-induced neuronal necrosis in the infant mouse hypothalamus: An electron microscopic study. *J. Neuropathol. Exp. Neurol.* 30: 75–90.
- ROTHMAN, S. M. (1983) Synaptic activity mediates death of hypoxic neurons. *Science* 220: 536–537.
- SYNTICHAKI, P. AND N. TAVERNARAKIS (2003) The biochemistry of neuronal necrosis: Rogue biology? *Nature Neurosci. Rev.* 4: 672–684.

tration of γ -hydroxybutyrate can cause euphoria, memory deficits, and unconsciousness. Presumably these effects arise from actions on GABAergic synapses in the CNS.

Inhibitory synapses employing GABA as their transmitter can exhibit three types of postsynaptic receptors, called GABA_A, GABA_B, and GABA_C. GABA_A and GABA_C receptors are ionotropic receptors, while GABA_B receptors are metabotropic. The ionotropic GABA receptors are usually



inhibitory because their associated channels are permeable to Cl^- (Figure 6.9A); the flow of the negatively charged chloride ions inhibits postsynaptic cells since the reversal potential for Cl^- is more negative than the threshold for neuronal firing (see Figure 5.19B). Like other ionotropic receptors, GABA receptors are pentamers assembled from a combination of five types of subunits (α , β , γ , δ , and ρ ; see Figure 6.4C). As a result of this subunit diversity, as well as variable stoichiometry of subunits, the function of GABA_A receptors differs widely among neuronal types. Drugs that act as agonists or modulators of postsynaptic GABA receptors, such as benzodiazepines and barbiturates, are used clinically to treat epilepsy and are effective sedatives and anesthetics. Binding sites for GABA, barbiturates, steroids, and picrotoxin are all located within the pore domain of the channel (Figure 6.9B). Another site, called the benzodiazepine binding site, lies outside the pore and modulates channel activity. Benzodiazepines, such as diazepam (Valium[®]) and chlordiazepoxide (Librium[®]), are tranquilizing (anxiety reducing) drugs that enhance GABAergic transmission by binding to the α and δ subunits of GABA_A receptors. Barbiturates, such as phenobarbital and pentobarbital, are hypnotics that bind to the α and β subunits of some GABA receptors and are used therapeutically for anesthesia and to control epilepsy. Another drug that can alter the activity of GABA-mediated inhibitory circuits is alcohol; at least some aspects of drunken behavior are caused by the alcohol-mediated alterations in ionotropic GABA receptors.

Metabotropic GABA receptors (GABA_B) are also widely distributed in brain. Like the ionotropic GABA_A receptors, GABA_B receptors are inhibitory. Rather than activating Cl^- selective channels, however, GABA_B-mediated inhibition is due to the activation of K^+ channels. A second mechanism for

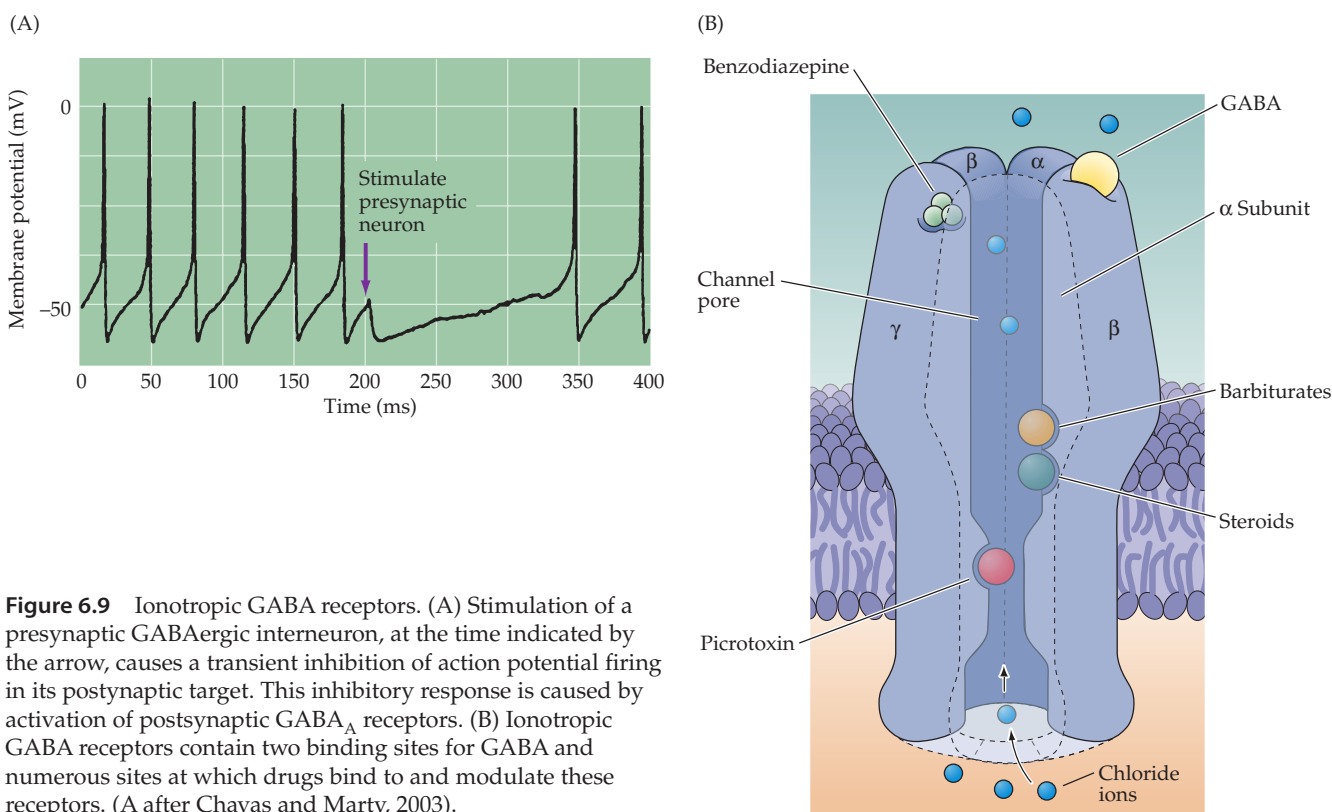
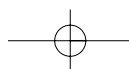


Figure 6.9 Ionotropic GABA receptors. (A) Stimulation of a presynaptic GABAergic interneuron, at the time indicated by the arrow, causes a transient inhibition of action potential firing in its postsynaptic target. This inhibitory response is caused by activation of postsynaptic GABA_A receptors. (B) Ionotropic GABA receptors contain two binding sites for GABA and numerous sites at which drugs bind to and modulate these receptors. (A after Chavas and Marty, 2003).



GABA_B-mediated inhibition is by blocking Ca²⁺ channels, which tends to hyperpolarize postsynaptic cells. Unlike most metabotropic receptors, GABA_B receptors appear to assemble as heterodimers of GABA_BR1 and R2 subunits.

The distribution of the neutral amino acid glycine in the central nervous system is more localized than that of GABA. About half of the inhibitory synapses in the spinal cord use glycine; most other inhibitory synapses use GABA. Glycine is synthesized from serine by the mitochondrial isoform of serine hydroxymethyltransferase (Figure 6.8B), and is transported into synaptic vesicles via the same vesicular inhibitory amino acid transporter that loads GABA into vesicles. Once released from the presynaptic cell, glycine is rapidly removed from the synaptic cleft by the plasma membrane glycine transporters. Mutations in the genes coding for some of these enzymes result in hyperglycinemia, a devastating neonatal disease characterized by lethargy, seizures, and mental retardation.

The receptors for glycine are also ligand-gated Cl⁻ channels, their general structure mirroring that of the GABA_A receptors. Glycine receptors are pentamers consisting of mixtures of the 4 gene products encoding glycine-binding α subunits, along with the accessory β subunit. Glycine receptors are potently blocked by strychnine, which may account for the toxic properties of this plant alkaloid (see Box B).

The Biogenic Amines

Biogenic amine transmitters regulate many brain functions and are also active in the peripheral nervous system. Because biogenic amines are implicated in such a wide range of behaviors (ranging from central homeostatic functions to cognitive phenomena such as attention), it is not surprising that defects in biogenic amines function are implicated in most psychiatric disorders. The pharmacology of amine synapses is critically important in psychotherapy, with drugs affecting the synthesis, receptor binding, or catabolism of these neurotransmitters being among the most important agents in the armamentarium of modern pharmacology (Box E). Many drugs of abuse also act on biogenic amine pathways.

There are five well-established biogenic amine neurotransmitters: the three catecholamines—dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline)—and histamine and serotonin (see Figure 6.1). All the catecholamines (so named because they share the catechol moiety) are derived from a common precursor, the amino acid tyrosine (Figure 6.10). The first step in catecholamine synthesis is catalyzed by tyrosine hydroxylase in a reaction requiring oxygen as a co-substrate and tetrahydrobiopterin as a cofactor to synthesize dihydroxyphenylalanine (DOPA). Histamine and serotonin are synthesized via other routes, as described below.

- *Dopamine* is present in several brain regions (Figure 6.11A), although the major dopamine-containing area of the brain is the corpus striatum, which receives major input from the substantia nigra and plays an essential role in the coordination of body movements. In Parkinson's disease, for instance, the dopaminergic neurons of the substantia nigra degenerate, leading to a characteristic motor dysfunction (see Box B in Chapter 17). Dopamine is also believed to be involved in motivation, reward, and reinforcement, and many drugs of abuse work by affecting dopaminergic synapses in the CNS (see Box A). In addition to these roles in the CNS, dopamine also plays a poorly understood role in some sympathetic ganglia.

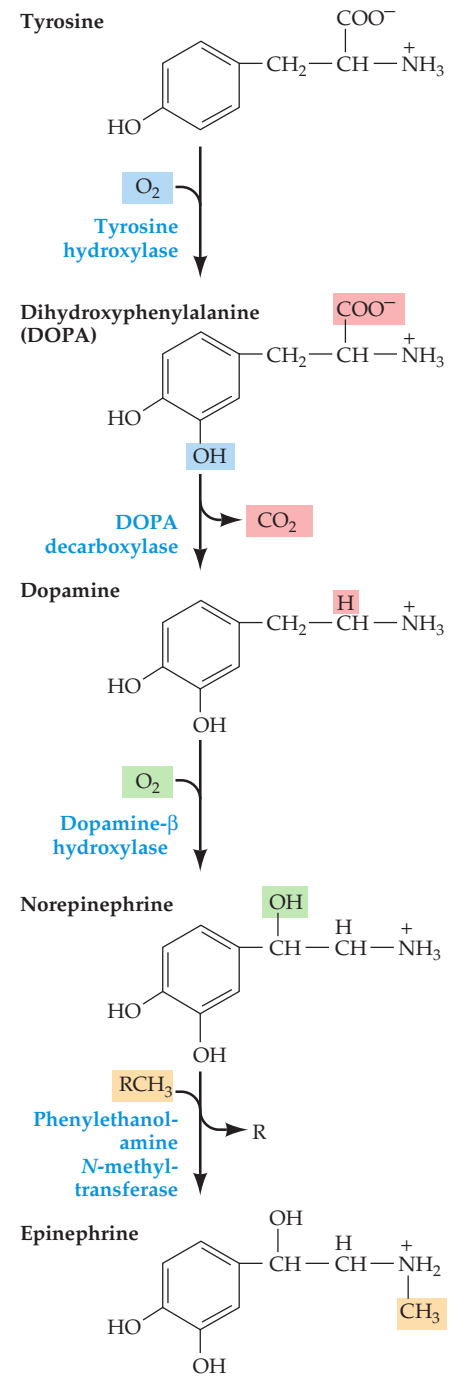
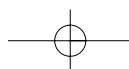


Figure 6.10 The biosynthetic pathway for the catecholamine neurotransmitters. The amino acid tyrosine is the precursor for all three catecholamines. The first step in this reaction pathway, catalyzed by tyrosine hydroxylase, is rate-limiting.



Box E

Biogenic Amine Neurotransmitters and Psychiatric Disorders

The regulation of the biogenic amine neurotransmitters is altered in a variety of psychiatric disorders. Indeed, most psychotropic drugs (defined as drugs that alter behavior, mood, or perception) selectively affect one or more steps in the synthesis, packaging, or degradation of biogenic amines. Sorting out how these drugs work has been extremely useful in beginning to understand the molecular mechanisms underlying some of these diseases.

Based on their effects on humans, psychotherapeutic drugs can be divided into several broad categories: antipsychotics, antianxiety drugs, antidepressants, and stimulants. The first antipsychotic drug used to ameliorate disorders such as schizophrenia was reserpine. Reserpine was developed in the 1950s and initially used as an antihypertensive agent; it blocks the uptake of norepinephrine into synaptic vesicles and therefore depletes the transmitter at aminergic terminals, diminishing the ability of the sympathetic division of the visceral motor system to cause vasoconstriction (see Chapter 20). A major side effect in hypertensive patients treated with reserpine—behavioral depression—suggested the possibility of using it as an antipsychotic agent in patients suffering from agitation and pathological anxiety. (Its ability to cause depression in mentally healthy individuals also suggested that aminergic transmitters are involved in mood disorders; see Box E in Chapter 28.)

Although reserpine is no longer used as an antipsychotic agent, its initial success stimulated the development of antipsychotic drugs such as chlorpromazine, haloperidol, and benperidol, which over the last several decades have radically changed the approach to treating psychotic disorders. Prior to the discovery of these drugs, psychotic patients were typically hospitalized for

long periods, sometimes indefinitely, and in the 1940s were subjected to desperate measures such as frontal lobotomy (see Box B in Chapter 25). Modern antipsychotic drugs now allow most patients to be treated on an outpatient basis after a brief hospital stay. Importantly, the clinical effectiveness of these drugs is correlated with their ability to block brain dopamine receptors, implying that activation of dopamine receptors contributes to some types of psychotic illness. A great deal of effort continues to be expended on developing more effective antipsychotic drugs with fewer side effects, and on discovering the mechanism and site of action of these medications.

The second category of psychotherapeutic drugs is the antianxiety agents. Anxiety disorders are estimated to afflict 10–35% of the population, making them the most common psychiatric problem. The two major forms of pathological anxiety—panic attacks and generalized anxiety disorder—both respond to drugs that affect aminergic transmission. The agents used to treat panic disorders include inhibitors of the enzyme monoamine oxidase (MAO inhibitors) required for the catabolism of the amine neurotransmitters, and blockers of serotonin receptors. The most effective drugs in treating generalized anxiety disorder have been benzodiazepines, such as chlordiazepoxide (Librium®), and diazepam (Valium®). In contrast to most other psychotherapeutic drugs, these agents increase the efficacy of transmission at GABA_A synapses rather than acting at aminergic synapses.

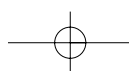
Antidepressants and stimulants also affect aminergic transmission. A large number of drugs are used clinically to treat depressive disorders. The three major classes of antidepressants—MAO inhibitors, tricyclic antidepressants, and

serotonin uptake blockers such as fluoxetine (Prozac®) and trazodone—all influence various aspects of aminergic transmission. MAO inhibitors such as phenelzine block the breakdown of amines, whereas the tricyclic antidepressants such as desipramine block the reuptake of norepinephrine and other amines. The extraordinarily popular antidepressant fluoxetine (Prozac®) selectively blocks the reuptake of serotonin without affecting the reuptake of catecholamines. Stimulants such as amphetamine are also used to treat some depressive disorders. Amphetamine stimulates the release of norepinephrine from nerve terminals; the transient “high” resulting from taking amphetamine may reflect the emotional opposite of the depression that sometimes follows reserpine-induced norepinephrine depletion.

Despite the relatively small number of aminergic neurons in the brain, this litany of pharmacological actions emphasizes that these neurons are critically important in the maintenance of mental health.

References

- FRANKLE, W. G., J. LERMA AND M. LARUELLE (2003) The synaptic hypothesis of schizophrenia. *Neuron* 39: 205–216.
- FREEDMAN, R. (2003) Schizophrenia. *N. Engl. J. Med.* 349: 1738–1749.
- LEWIS, D. A. AND P. LEVITT (2002) Schizophrenia as a disorder of neurodevelopment. *Annu. Rev. Neurosci.* 25: 409–432.
- NESTLER, E. J., M. BARROT, R. J. DiLEONE, A. J. EISCH, S. J. GOLD AND L. M. MONTEGGIA (2002) Neurobiology of depression. *Neuron* 34: 13–25.



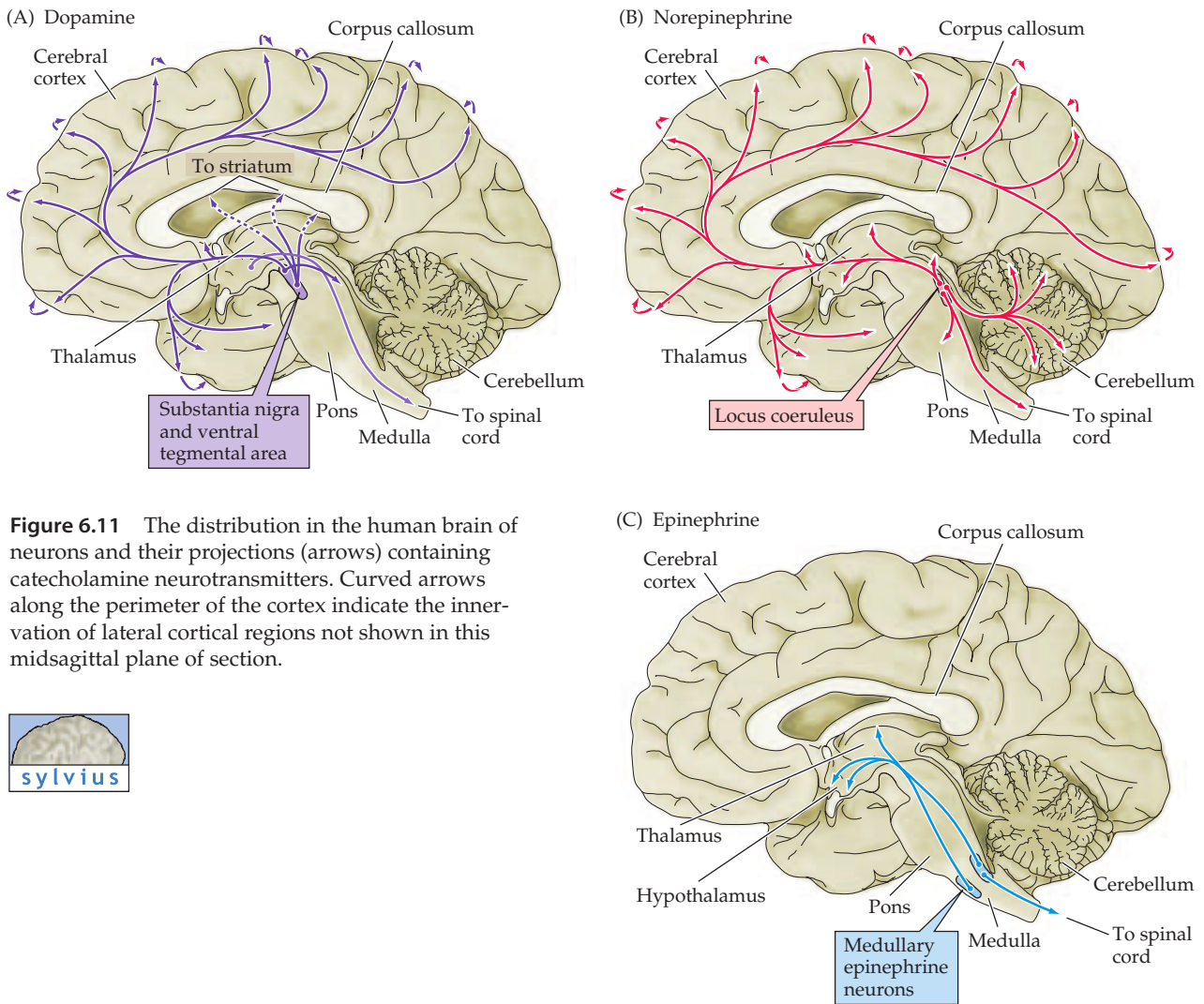
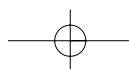


Figure 6.11 The distribution in the human brain of neurons and their projections (arrows) containing catecholamine neurotransmitters. Curved arrows along the perimeter of the cortex indicate the innervation of lateral cortical regions not shown in this midsagittal plane of section.



Dopamine is produced by the action of DOPA decarboxylase on DOPA (see Figure 6.10). Following its synthesis in the cytoplasm of presynaptic terminals, dopamine is loaded into synaptic vesicles via a vesicular monoamine transporter (VMAT). Dopamine action in the synaptic cleft is terminated by reuptake of dopamine into nerve terminals or surrounding glial cells by a Na^+ -dependent dopamine transporter, termed DAT. Cocaine apparently produces its psychotropic effects by binding to and inhibiting DAT, yielding a net increase in dopamine release from specific brain areas. Amphetamine, another addictive drug, also inhibits DAT as well as the transporter for norepinephrine (see below). The two major enzymes involved in the catabolism of dopamine are monoamine oxidase (MAO) and catechol *O*-methyltransferase (COMT). Both neurons and glia contain mitochondrial MAO and cytoplasmic COMT. Inhibitors of these enzymes, such as phenelzine and tranylcypromine, are used clinically as antidepressants (see Box E).

Once released, dopamine acts exclusively by activating G-protein-coupled receptors. These are mainly dopamine-specific receptors, although β -adrenergic receptors also serve as important targets of norepinephrine and epinephrine (see below). Most dopamine receptor subtypes (see Figure 6.5B)



act by either activating or inhibiting adenylyl cyclase (see Chapter 7). Activation of these receptors generally contribute to complex behaviors; for example, administration of dopamine receptor agonists elicits hyperactivity and repetitive, stereotyped behavior in laboratory animals. Activation of another type of dopamine receptor in the medulla inhibits vomiting. Thus, antagonists of these receptors are used as emetics to induce vomiting after poisoning or a drug overdose. Dopamine receptor antagonists can also elicit catalepsy, a state in which it is difficult to initiate voluntary motor movement, suggesting a basis for this aspect of some psychoses.

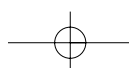
- *Norepinephrine* (also called noradrenaline) is used as a neurotransmitter in the locus coeruleus, a brainstem nucleus that projects diffusely to a variety of forebrain targets (Figure 6.11B) and influences sleep and wakefulness, attention, and feeding behavior. Perhaps the most prominent noradrenergic neurons are sympathetic ganglion cells, which employ norepinephrine as the major peripheral transmitter in this division of the visceral motor system (see Chapter 20).

Norepinephrine synthesis requires dopamine β -hydroxylase, which catalyzes the production of norepinephrine from dopamine (see Figure 6.10). Norepinephrine is then loaded into synaptic vesicles via the same VMAT involved in vesicular dopamine transport. Norepinephrine is cleared from the synaptic cleft by the norepinephrine transporter (NET), which also is capable of taking up dopamine. As mentioned, NET serves as a molecular target of amphetamine, which acts as a stimulant by producing a net increase in the release of norepinephrine and dopamine. A mutation in the NET gene is a cause of orthostatic intolerance, a disorder that produces lightheadedness while standing up. Like dopamine, norepinephrine is degraded by MAO and COMT.

Norepinephrine, as well as epinephrine, acts on α - and β -adrenergic receptors (Figure 6.5B). Both types of receptor are G-protein-coupled; in fact, the β -adrenergic receptor was the first identified metabotropic neurotransmitter receptor. Two subclasses of α -adrenergic receptors are now known. Activation of α_1 receptors usually results in a slow depolarization linked to the inhibition of K^+ channels, while activation of α_2 receptors produces a slow hyperpolarization due to the activation of a different type of K^+ channel. There are three subtypes of β -adrenergic receptor, two of which are expressed in many types of neurons. Agonists and antagonists of adrenergic receptors, such as the β blocker propranolol (Inderol[®]), are used clinically for a variety of conditions ranging from cardiac arrhythmias to migraine headaches. However, most of the actions of these drugs are on smooth muscle receptors, particularly in the cardiovascular and respiratory systems (see Chapter 20).

- *Epinephrine* (also called adrenaline) is found in the brain at lower levels than the other catecholamines and also is present in fewer brain neurons than other catecholamines. Epinephrine-containing neurons in the central nervous system are primarily in the lateral tegmental system and in the medulla and project to the hypothalamus and thalamus (Figure 6.11C). The function of these epinephrine-secreting neurons is not known.

The enzyme that synthesizes epinephrine, phenylethanolamine-*N*-methyltransferase (see Figure 6.10), is present only in epinephrine-secreting neurons. Otherwise, the metabolism of epinephrine is very similar to that of norepinephrine. Epinephrine is loaded into vesicles via the VMAT. No plasma membrane transporter specific for epinephrine has been identified, though the NET is capable of transporting epinephrine. As already noted, epinephrine acts on both α - and β -adrenergic receptors.



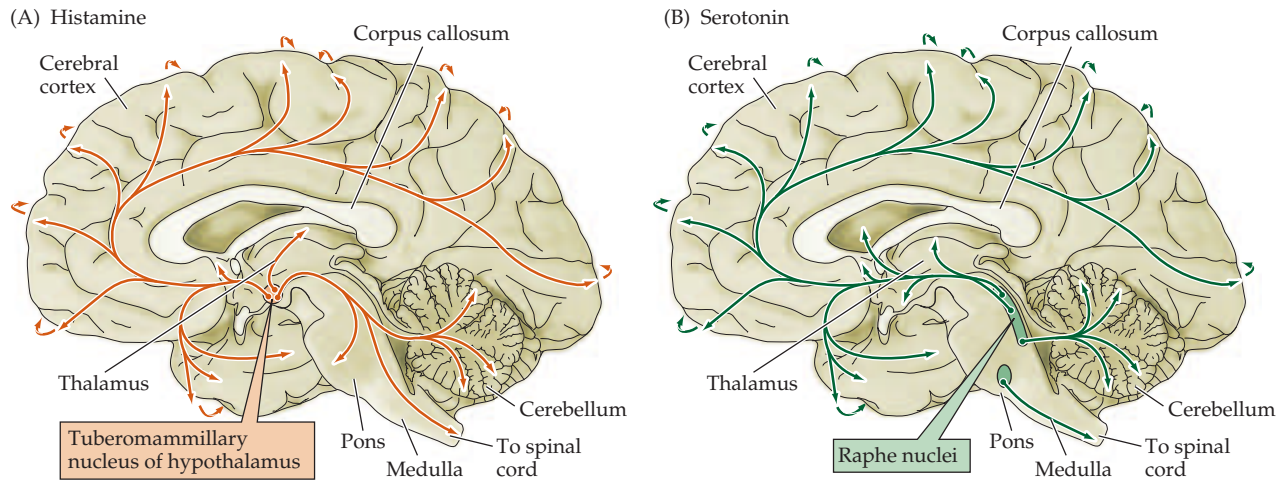
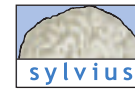


Figure 6.12 The distribution in the human brain of neurons and their projections (arrows) containing histamine (A) or serotonin (B). Curved arrows along the perimeter of the cortex indicate the innervation of lateral cortical regions not shown in this midsagittal plane of section.



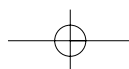
- *Histamine* is found in neurons in the hypothalamus that send sparse but widespread projections to almost all regions of the brain and spinal cord (Figure 6.12A). The central histamine projections mediate arousal and attention, similar to central ACh and norepinephrine projections. Histamine also controls the reactivity of the vestibular system. Allergic reactions or tissue damage cause release of histamine from mast cells in the bloodstream. The close proximity of mast cells to blood vessels, together with the potent actions of histamine on blood vessels, also raises the possibility that histamine may influence brain blood flow.

Histamine is produced from the amino acid histidine by a histidine decarboxylase (Figure 6.13A) and is transported into vesicles via the same VMAT as the catecholamines. No plasma membrane histamine transporter has been identified yet. Histamine is degraded by the combined actions of histamine methyltransferase and MAO.

There are three known types of histamine receptors, all of which are G-protein-coupled receptors (Figure 6.5B). Because of the importance of histamine receptors in the mediation of allergic responses, many histamine receptor antagonists have been developed as antihistamine agents. Antihistamines that cross the blood-brain barrier, such as diphenhydramine (Benadryl[®]), act as sedatives by interfering with the roles of histamine in CNS arousal. Antagonists of the H₁ receptor also are used to prevent motion sickness, perhaps because of the role of histamine in controlling vestibular function. H₂ receptors control the secretion of gastric acid in the digestive system, allowing H₂ receptor antagonists to be used in the treatment of a variety of upper gastrointestinal disorders (e.g., peptic ulcers).

- *Serotonin*, or 5-hydroxytryptamine (5-HT), was initially thought to increase vascular tone by virtue of its presence in serum (hence the name serotonin). Serotonin is found primarily in groups of neurons in the raphe region of the pons and upper brainstem, which have widespread projections to the forebrain (see Figure 6.12B) and regulate sleep and wakefulness (see Chapter 27). 5-HT occupies a place of prominence in neuropharmacology because a large number of antipsychotic drugs that are valuable in the treatment of depression and anxiety act on serotonergic pathways (see Box E).

5-HT is synthesized from the amino acid tryptophan, which is an essential dietary requirement. Tryptophan is taken up into neurons by a plasma mem-



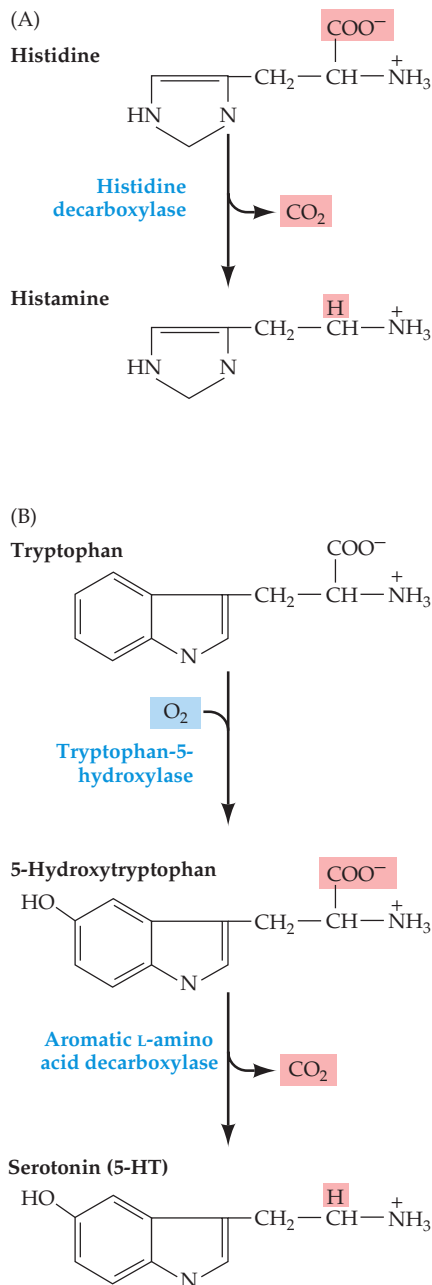


Figure 6.13 Synthesis of histamine and serotonin. (A) Histamine is synthesized from the amino acid histidine. (B) Serotonin is derived from the amino acid tryptophan by a two-step process that requires the enzymes tryptophan-5-hydroxylase and a decarboxylase.

brane transporter and hydroxylated in a reaction catalyzed by the enzyme tryptophan-5-hydroxylase (Figure 6.13B), the rate-limiting step for 5-HT synthesis. Loading of 5-HT into synaptic vesicles is done by the VMAT that is also responsible for loading of other monoamines into synaptic vesicles. The synaptic effects of serotonin are terminated by transport back into nerve terminals via a specific serotonin transporter (SERT). Many antidepressant drugs are selective serotonin reuptake inhibitors (SSRIs) that inhibit transport of 5-HT by SERT. Perhaps the best known example of an SSRI is Prozac (see Box E). The primary catabolic pathway for 5-HT is mediated by MAO.

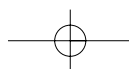
A large number of 5-HT receptors have been identified. Most 5-HT receptors are metabotropic (see Figure 6.5B). These have been implicated in behaviors, including the emotions, circadian rhythms, motor behaviors, and state of mental arousal. Impairments in the function of these receptors have been implicated in numerous psychiatric disorders, such as depression, anxiety disorders, and schizophrenia (see Chapter 28), and drugs acting on serotonin receptors are effective treatments for a number of these conditions. Activation of 5-HT receptors also mediates satiety and decreased food consumption, which is why serotonergic drugs are sometimes useful in treating eating disorders.

Only one group of serotonin receptors, called the 5-HT₃ receptors, are ligand-gated ion channels (see Figure 6.4C). These are non-selective cation channels and therefore mediate excitatory postsynaptic responses. Their general structure, with functional channels formed by assembly of multiple subunits, is similar to the other ionotropic receptors described in the chapter. Two types of 5-HT₃ subunit are known, and form functional channels by assembling as a heteromultimer. 5-HT receptors are targets for a wide variety of therapeutic drugs including ondansetron (Zofran[®]) and granisetron (Kytril[®]), which are used to prevent postoperative nausea and chemotherapy-induced emesis.

ATP and Other Purines

Interestingly, all synaptic vesicles contain ATP, which is co-released with one or more “classical” neurotransmitters. This observation raises the possibility that ATP acts as a co-transmitter. It has been known since the 1920s that the extracellular application of ATP (or its breakdown products AMP and adenosine) can elicit electrical responses in neurons. The idea that some purines (so named because all these compounds contain a purine ring; see Figure 6.1) are also neurotransmitters has now received considerable experimental support. ATP acts as an excitatory neurotransmitter in motor neurons of the spinal cord, as well as sensory and autonomic ganglia. Postsynaptic actions of ATP have also been demonstrated in the central nervous system, specifically for dorsal horn neurons and in a subset of hippocampal neurons. Adenosine, however, cannot be considered a classical neurotransmitter because it is not stored in synaptic vesicles or released in a Ca²⁺-dependent manner. Rather, it is generated from ATP by the action of extracellular enzymes. A number of enzymes, such as apyrase and ecto-5′ nucleotidase, as well as nucleoside transporters are involved in the rapid catabolism and removal of purines from extracellular locations. Despite the relative novelty of this evidence, it suggests that excitatory transmission via purinergic synapses is widespread in the mammalian brain.

In accord with this evidence, receptors for both ATP and adenosine are widely distributed in the nervous system, as well as many other tissues.



Three classes of these purinergic receptors are now known. One of these classes consists of ligand-gated ion channels (see Figure 6.4C); the other two are G-protein-coupled metabotropic receptors (see Figure 6.5B). Like many ionotropic transmitter receptors, the ligand-gated channels are nonselective cation channels that mediate excitatory postsynaptic responses. The genes encoding these channels, however, are unique in that they appear to have only two transmembrane domains. Ionotropic purinergic receptors are widely distributed in central and peripheral neurons. In sensory nerves they evidently play a role in mechanosensation and pain; their function in most other cells, however, is not known.

The two types of metabotropic receptors activated by purines differ in their sensitivity to agonists: One type is preferentially stimulated by adenosine, whereas the other is preferentially activated by ATP. Both receptor types are found throughout the brain, as well as in peripheral tissues such as the heart, adipose tissue, and the kidney. Xanthines such as caffeine and theophylline block adenosine receptors, and this activity is thought to be responsible for the stimulant effects of these agents.

Peptide Neurotransmitters

Many peptides known to be hormones also act as neurotransmitters. Some peptide transmitters have been implicated in modulating emotions (see Chapter 28). Others, such as substance P and the opioid peptides, are involved in the perception of pain (see Chapter 9). Still other peptides, such as melanocyte-stimulating hormone, adrenocorticotropin, and β -endorphin, regulate complex responses to stress.

The mechanisms responsible for the synthesis and packaging of peptide transmitters are fundamentally different from those used for the small-molecule neurotransmitters and are much like the synthesis of proteins that are secreted from non-neuronal cells (pancreatic enzymes, for instance). Peptide-secreting neurons generally synthesize polypeptides in their cell bodies that are much larger than the final, “mature” peptide. Processing these polypeptides in their cell bodies, which are called **pre-propeptides** (or pre-proproteins), takes place by a sequence of reactions in several intracellular organelles. Pre-propeptides are synthesized in the rough endoplasmic reticulum, where the signal sequence of amino acids—that is, the sequence indicating that the peptide is to be secreted—is removed. The remaining polypeptide, called a **propeptide** (or proprotein), then traverses the Golgi apparatus and is packaged into vesicles in the *trans*-Golgi network. The final stages of peptide neurotransmitter processing occur after packaging into vesicles and involve proteolytic cleavage, modification of the ends of the peptide, glycosylation, phosphorylation, and disulfide bond formation.

Propeptide precursors are typically larger than their active peptide products and can give rise to more than one species of neuropeptide (Figure 6.14). The means that multiple neuroactive peptides can be released from a single vesicle. In addition, neuropeptides often are co-released with small-molecule neurotransmitters. Thus, peptidergic synapses often elicit complex postsynaptic responses. Peptides are catabolized into inactive amino acid fragments by enzymes called peptidases, usually located on the extracellular surface of the plasma membrane.

The biological activity of the peptide neurotransmitters depends on their amino acid sequence (Figure 6.15). Based on their amino acid sequences, neuropeptide transmitters have been loosely grouped into five categories:

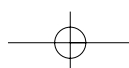
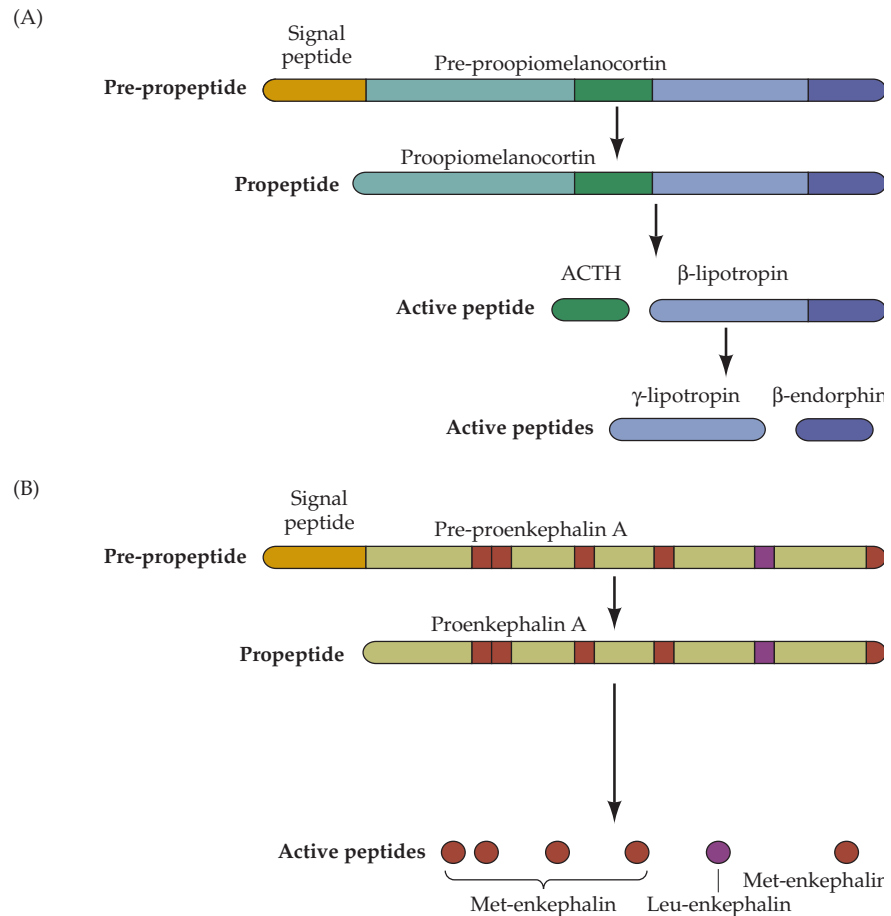


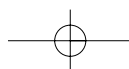
Figure 6.14 Proteolytic processing of the pre-peptides pre-proopiomelanocortin (A) and pre-proenkephalin A (B). For each pre-peptide, the signal sequence is indicated in orange at the left; the locations of active peptide products are indicated by different colors. The maturation of the pre-peptides involves cleaving the signal sequence and other proteolytic processing. Such processing can result in a number of different neuroactive peptides such as ACTH, γ -lipotropin, and β -endorphin (A), or multiple copies of the same peptide, such as met-enkephalin (B).



the brain/gut peptides, opioid peptides, pituitary peptides, hypothalamic releasing hormones, and a catch-all category containing other peptides that are not easily classified.

Substance P is an example of the first of these categories (Figure 6.15A). The study of neuropeptides actually began more than 60 years ago with the accidental discovery of substance P, a powerful hypotensive agent. (The peculiar name derives from the fact that this molecule was an unidentified component of *powder* extracts from brain and intestine.) This 11-amino-acid peptide (see Figure 6.15) is present in high concentrations in the human hippocampus, neocortex, and also in the gastrointestinal tract; hence its classification as a brain/gut peptide. It is also released from C fibers, the small-diameter afferents in peripheral nerves that convey information about pain and temperature (as well as postganglionic autonomic signals). Substance P is a sensory neurotransmitter in the spinal cord, where its release can be inhibited by opioid peptides released from spinal cord interneurons, resulting in the suppression of pain (see Chapter 9). The diversity of neuropeptides is highlighted by the finding that the gene coding for substance P encodes a number of other neuroactive peptides including neurokinin A, neuropeptide K, and neuropeptide γ .

An especially important category of peptide neurotransmitters is the family of opioids (Figure 6.15B). These peptides are so named because they bind



(A) Brain–gut peptides



(B) Opioid peptides



Amino acid properties

- Hydrophobic
- Polar, uncharged
- Acidic
- Basic

(C) Pituitary peptides



(D) Hypothalamic–releasing peptides



(E) Miscellaneous peptides



Figure 6.15 Neuropeptides vary in length, but usually contain between 3 and 36 amino acids. The sequence of amino acids determines the biological activity of each peptide.

to the same postsynaptic receptors activated by opium. The opium poppy has been cultivated for at least 5000 years, and its derivatives have been used as an analgesic since at least the Renaissance. The active ingredients in opium are a variety of plant alkaloids, predominantly morphine. Morphine, named for Morpheus, the Greek god of dreams, is still one of the most effective analgesics in use today, despite its addictive potential (see Box A). Synthetic opiates such as meperidine and methadone are also used as analgesics, and fentanyl, a drug with 80 times the analgesic potency of morphine, is widely used in clinical anesthesiology.

The opioid peptides were discovered in the 1970s during a search for endorphins, *endogenous* compounds that mimicked the actions of *morphine*. It was hoped that such compounds would be analgesics, and that understanding them would shed light on drug addiction. The endogenous ligands of the opioid receptors have now been identified as a family of more than 20 opioid peptides that fall into three classes: the endorphins, the enkephalins, and the dynorphins (Table 6.2). Each of these classes are liberated from an inactive pre-propeptide (pre-proopiomelanocortin, pre-proenkephalin A, and pre-prodynorphin), derived from distinct genes (see Figure 6.14). Opioid precursor processing is carried out by tissue-specific processing enzymes that are packaged into vesicles, along with the precursor peptide, in the Golgi apparatus.

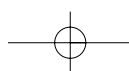


TABLE 6.2
Endogenous Opioid Peptides

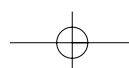
<i>Name</i>	<i>Amino acid sequence^a</i>
Endorphins	
α -Endorphin	<i>Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr</i>
α -Neoendorphin	<i>Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys</i>
β -Endorphin	<i>Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Val-Lys-Asn-Ala-His-Lys-Gly-Gln</i>
γ -Endorphin	<i>Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu</i>
Enkephalins	
Leu-enkephalin	<i>Tyr-Gly-Gly-Phe-Leu</i>
Met-enkephalin	<i>Tyr-Gly-Gly-Phe-Met</i>
Dynorphins	
Dynorphin A	<i>Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln</i>
Dynorphin B	<i>Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr</i>

^aNote the initial homology, indicated by italics.

Opioid peptides are widely distributed throughout the brain and are often co-localized with other small-molecule neurotransmitters, such as GABA and 5-HT. In general, these peptides tend to be depressants. When injected intracerebrally in experimental animals, they act as analgesics; on the basis of this and other evidence, opioids are likely to be involved in the mechanisms underlying acupuncture-induced analgesia. Opioids are also involved in complex behaviors such as sexual attraction and aggressive/submissive behaviors. They have also been implicated in psychiatric disorders such as schizophrenia and autism, although the evidence for this is debated. Unfortunately, the repeated administration of opioids leads to tolerance and addiction.

Virtually all neuropeptides initiate their effects by activating G-protein-coupled receptors. The study of these metabotropic peptide receptors in the brain has been difficult because few specific agonists and antagonists are known. Peptides activate their receptors at low (nM to μ M) concentrations compared to the concentrations required to activate receptors for small-molecule neurotransmitters. These properties allow the postsynaptic targets of peptides to be quite far removed from presynaptic terminals and to modulate the electrical properties of neurons that are simply in the vicinity of the site of peptide release. Neuropeptide receptor activation is especially important in regulating the postganglionic output from sympathetic ganglia and the activity of the gut (see Chapter 20). Peptide receptors, particularly the neuropeptide Y receptor, are also implicated in the initiation and maintenance of feeding behavior leading to satiety or obesity.

Other behaviors ascribed to peptide receptor activation include anxiety and panic attacks, and antagonists of cholecystokinin receptors are clinically useful in the treatment of these afflictions. Other useful drugs have been developed by targeting the opiate receptors. Three well-defined opioid receptor subtypes (μ , δ , and κ) play a role in reward mechanisms as well as addiction. The μ -opiate receptor has been specifically identified as the primary site for drug reward mediated by opiate drugs



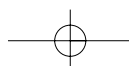
Unconventional Neurotransmitters

In addition to the conventional neurotransmitters already described, some unusual molecules are also used for signaling between neurons and their targets. These chemical signals can be considered as neurotransmitters because of their roles in interneuronal signaling and because their release from neurons is regulated by Ca^{2+} . However, they are unconventional, in comparison to other neurotransmitters, because they are not stored in synaptic vesicles and are not released from presynaptic terminals via exocytotic mechanisms. In fact, these unconventional neurotransmitters need not be released from presynaptic terminals at all and are often associated with “retrograde” signaling from postsynaptic cells back to presynaptic terminals.

- *Endocannabinoids* are a family of related endogenous signals that interact with cannabinoid receptors. These receptors are the molecular targets of Δ^9 -tetrahydrocannabinol, the psychoactive component of the marijuana plant, *Cannabis* (Box F). While some members of this emerging group of chemical signals remain to be determined, anandamide and 2-arachidonylglycerol (2-AG) have been established as endocannabinoids. These signals are unsaturated fatty acid with polar head groups and are produced by enzymatic degradation of membrane lipids (Figure 6.16A,B). Production of endocannabinoids is stimulated by a second messenger signal within postsynaptic neurons, typically a rise in postsynaptic Ca^{2+} concentration. Although the mechanism of endocannabinoid release is not entirely clear, it is likely that these hydrophobic signals diffuse through the postsynaptic membrane to reach cannabinoid receptors on other nearby cells. Endocannabinoid action is terminated by carrier-mediated transport of these signals back into the postsynaptic neuron. There they are hydrolyzed by the enzyme fatty acid hydrolase (FAAH).

At least two types of cannabinoid receptor have been identified, with most actions of endocannabinoids in the CNS mediated by the type termed CB1. CB1 is a G-protein-coupled receptor that is related to the metabotropic receptors for ACh, glutamate, and the other conventional neurotransmitters. Several compounds that are structurally related to endocannabinoids and that bind to the CB1 receptor have been synthesized (see Figure 6.16C). These compounds act as agonists or antagonists of the CB1 receptor and serve as both tools for elucidating the physiological functions of endocannabinoids and as targets for developing therapeutically useful drugs.

Endocannabinoids participate in several forms of synaptic regulation. The best-documented action of these agents is to inhibit communication between postsynaptic target cells and their presynaptic inputs. In both the hippocampus and the cerebellum, among other regions, endocannabinoids serve as retrograde signals to regulate GABA release at certain inhibitory terminals. At such synapses, depolarization of the postsynaptic neuron causes a transient reduction in inhibitory postsynaptic responses (Figure 6.17). Depolarization reduces synaptic transmission by elevating the concentration of Ca^{2+} within the postsynaptic neuron. This rise in Ca^{2+} triggers synthesis and release of endocannabinoids from the postsynaptic cells. The endocannabinoids then make their way to the presynaptic terminals and bind to CB1 receptors on these terminals. Activation of the CB1 receptors inhibits the amount of GABA released in response to presynaptic action potentials, thereby reducing inhibitory transmission. These mechanisms responsible for the reduction in GABA release are not entirely clear, but probably involve effects on voltage-gated Ca^{2+} channels and/or K^+ channels in the presynaptic neurons.



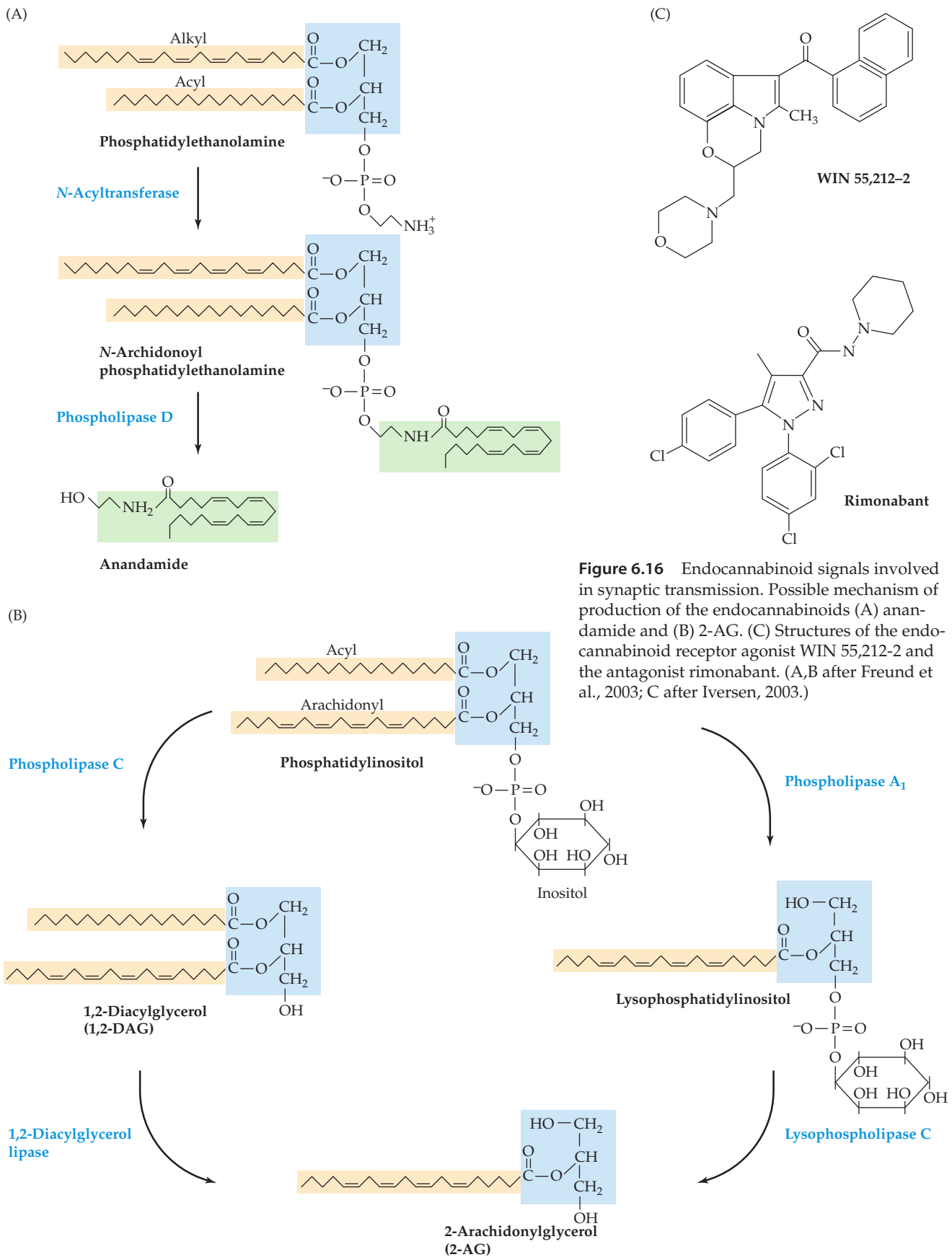
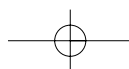


Figure 6.16 Endocannabinoid signals involved in synaptic transmission. Possible mechanism of production of the endocannabinoids (A) anandamide and (B) 2-AG. (C) Structures of the endocannabinoid receptor agonist WIN 55,212-2 and the antagonist rimonabant. (A,B after Freund et al., 2003; C after Iversen, 2003.)



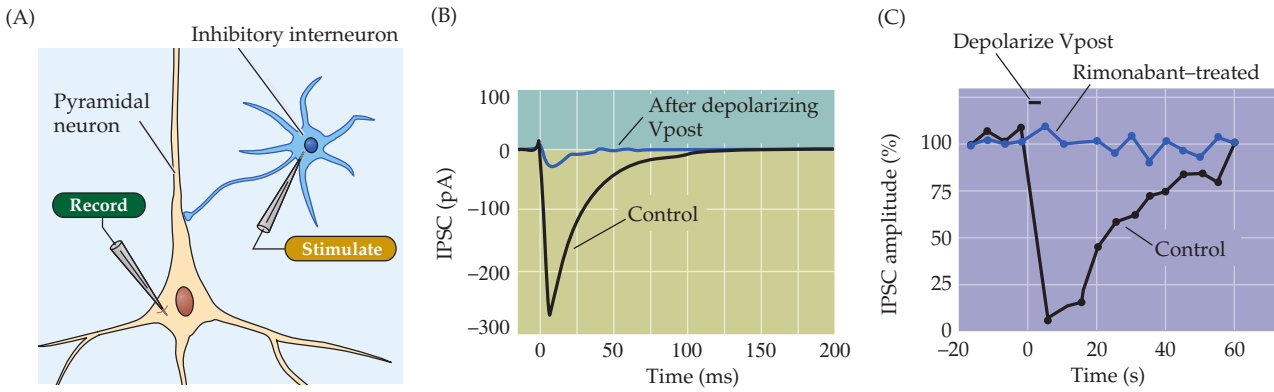
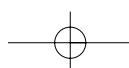
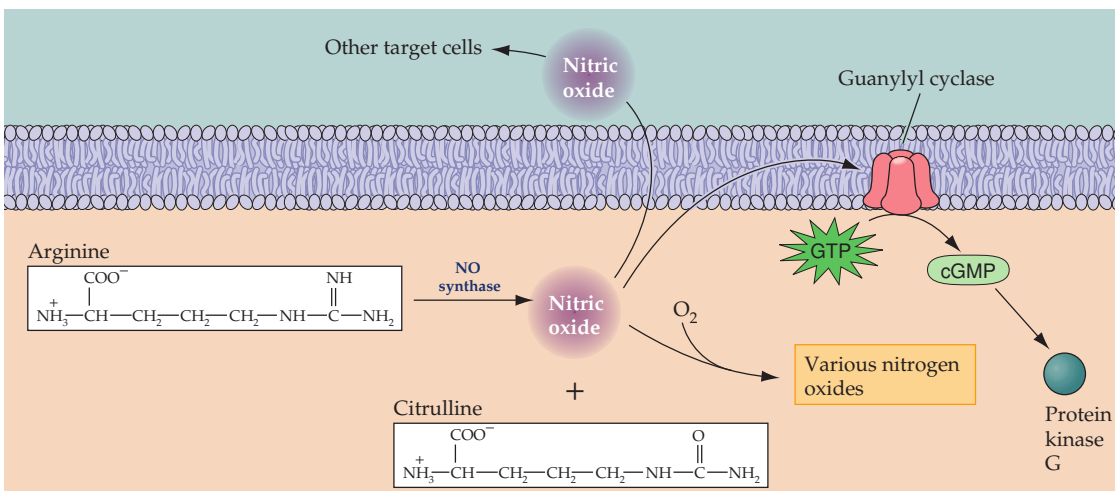


Figure 6.17 Endocannabinoid-mediated retrograde control of GABA release. (A) Experimental arrangement. Stimulation of a presynaptic interneuron causes release of GABA onto a postsynaptic pyramidal neuron. (B) IPSCs elicited by the inhibitory synapse (control) are reduced in amplitude following a brief depolarization of the postsynaptic neuron. This reduction in the IPSC is due to less GABA being released from the presynaptic interneuron. (C) The reduction in IPSC amplitude produced by postsynaptic depolarization lasts a few seconds and is mediated by endocannabinoids, because it is prevented by the endocannabinoid receptor antagonist rimonabant. (B,C after Ohno-Shosaku et al., 2001.)

• *Nitric oxide* (NO) is an unusual but especially interesting chemical signal. NO is a gas that is produced by the action of nitric oxide synthase, an enzyme that converts the amino acid arginine into a metabolite (citrulline) and simultaneously generates NO (Figure 6.18). NO is produced by an enzyme, nitric oxide synthase. Neuronal NO synthase is regulated by Ca^{2+} binding to the Ca^{2+} sensor protein calmodulin (see Chapter 7). Once produced, NO can permeate the plasma membrane, meaning that NO generated inside one cell can travel through the extracellular medium and act within nearby cells. Thus, this gaseous signal has a range of influence that extends well beyond the cell of origin, diffusing a few tens of micrometers from its site of production before it is degraded. This property makes NO a

Figure 6.18 Synthesis, release, and termination of NO.



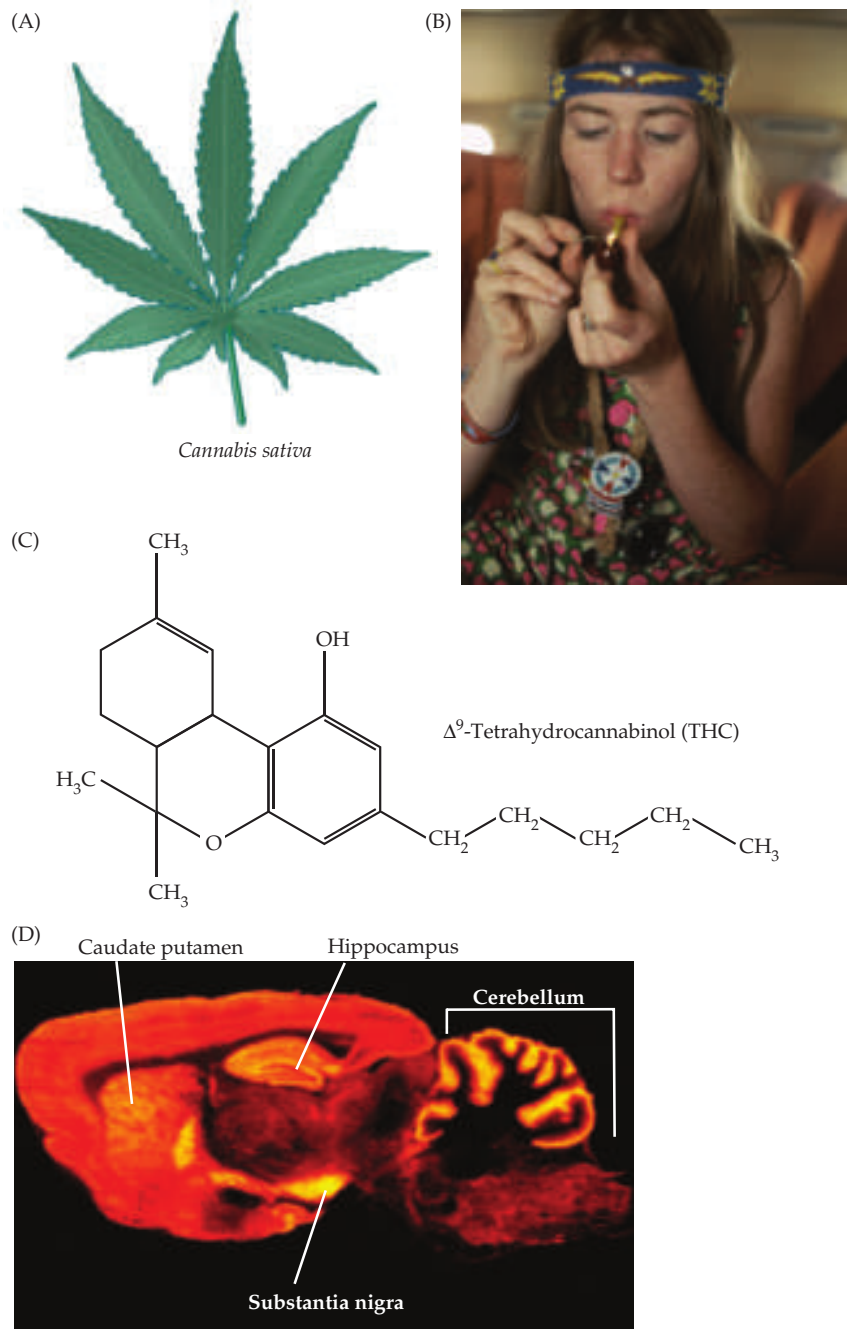
Box F

Marijuana and the Brain

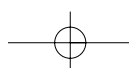
Medicinal use of the marijuana plant, *Cannabis sativa* (Figure A), dates back thousands of years. Ancient civilizations—including both Greek and Roman societies in Europe, as well as Indian and Chinese cultures in Asia—appreciated that this plant was capable of producing relaxation, euphoria, and a number of other psychopharmacological actions. In more recent times, medicinal use of marijuana has largely subsided (although it remains useful in relieving the symptoms of terminal cancer patients); the recreational use of marijuana (Figure B) has become so popular that some societies have decriminalized its use.

Understanding the brain mechanisms underlying the actions of marijuana was advanced by the discovery that a cannabinoid, Δ^9 -tetrahydrocannabinol (THC; Figure C), is the active component of marijuana. This finding led to the development of synthetic derivatives, such as WIN 55,212-2 and rimonabant (see Figure 6.16), that have served as valuable tools for probing the brain actions of THC. Of particular interest is that receptors for these cannabinoids exist in the brain and exhibit marked regional variations in distribution, being especially enriched in the brain areas—such as substantia nigra and caudate putamen—that have been implicated in drug abuse (Figure D). The presence of these brain receptors for cannabinoids led in turn to a search for endogenous cannabinoid compounds in the brain, culminating in the discovery of endocannabinoids such as 2-AG and anandamide (see Figure 6.16). This path of discovery closely parallels the identification of endogenous opioid peptides, which resulted from the search for endogenous morphine-like compounds in the brain (see text and Table 6.2).

Given that THC interacts with brain endocannabinoid receptors, particularly



(A) Leaf of *Cannabis sativa*, the marijuana plant. (B) Smoking ground-up *Cannabis* leaves is a popular method of achieving the euphoric effects of marijuana. (C) Structure of THC (Δ^9 -tetrahydrocannabinol), the active ingredient of marijuana. (D) Distribution of brain CB1 receptors, visualized by examining the binding of CP-55,940, a CB1 receptor ligand. (B photo © Henry Diltz/Corbis; C after Iversen, 2003; D courtesy of M. Herkenham, NIMH.)



the CB1 receptor, it is likely that such actions are responsible for the behavioral consequences of marijuana use. Indeed, many of the well-documented effects of marijuana are consistent with the distribution and actions of brain CB1 receptors. For example, marijuana effects on perception could be due to CB1 receptors in the neocortex, effects on psychomotor control due to endocannabinoid receptors in the basal ganglia and cerebellum, effects on short-term memory due to cannabinoid

receptors in the hippocampus, and the well-known effects of marijuana on stimulating appetite due to hypothalamic actions. While formal links between these behavioral consequences of marijuana and the underlying brain mechanisms are still being forged, studies of the actions of this drug have shed substantial light on basic synaptic mechanisms, which promise to further elucidate the mode of action of one of the world's most popular drugs.

References

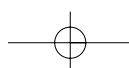
- ADAMS, A. R. (1941) Marihuana. *Harvey Lect.* 37: 168–168.
- FREUND, T. F., I. KATONA AND D. PIOMELLI (2003) Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev.* 83: 1017–1066.
- GERDEMAN, G. L., J. G. PARTRIDGE, C. R. LUPICA AND D. M. LOVINGER (2003) It could be habit forming: Drugs of abuse and striatal synaptic plasticity. *Trends Neurosci.* 26: 184–192.
- IVERSEN, L. (2003) Cannabis and the brain. *Brain* 126: 1252–1270.
- MECHOULAM, R. (1970) Marihuana chemistry. *Science* 168: 1159–1166.

potentially useful agent for coordinating the activities of multiple cells in a very localized region and may mediate certain forms of synaptic plasticity that spread within small networks of neurons.

All of the known actions of NO are mediated within its cellular targets; for this reason, NO often is considered a second messenger rather than a neurotransmitter. Some of these actions of NO are due to the activation of the enzyme guanylyl cyclase, which then produces the second messenger cGMP within target cells (see Chapter 7). Other actions of NO are the result of covalent modification of target proteins via nitrosylation, the addition of a nitryl group to selected amino acids within the proteins. NO decays spontaneously by reacting with oxygen to produce inactive nitrogen oxides. As a result, NO signals last for only a short time, on the order of seconds or less. NO signaling evidently regulates a variety of synapses that also employ conventional neurotransmitters; so far, presynaptic terminals that release glutamate are the best-studied target of NO in the central nervous system. NO may also be involved in some neurological diseases. For example, it has been proposed that an imbalance between nitric oxide and superoxide generation underlies some neurodegenerative diseases.

Summary

The complex synaptic computations occurring at neural circuits throughout the brain arise from the actions of a large number of neurotransmitters, which act on an even larger number of postsynaptic neurotransmitter receptors. Glutamate is the major excitatory neurotransmitter in the brain, whereas GABA and glycine are the major inhibitory neurotransmitters. The actions of these small-molecule neurotransmitters are typically faster than those of the neuropeptides. Thus, most small-molecule transmitters mediate synaptic transmission when a rapid response is essential, whereas the neuropeptide transmitters, as well as the biogenic amines and some small-molecule neurotransmitters, tend to modulate ongoing activity in the brain or in peripheral target tissues in a more gradual and ongoing way. Two broadly different families of neurotransmitter receptors have evolved to carry out the postsynaptic signaling actions of neurotransmitters. Ionotropic or ligand-



gated ion channels combine the neurotransmitter receptor and ion channel in one molecular entity, and therefore give rise to rapid postsynaptic electrical responses. Metabotropic receptors regulate the activity of postsynaptic ion channels indirectly, usually via G-proteins, and induce slower and longer-lasting electrical responses. Metabotropic receptors are especially important in regulating behavior, and drugs targeting these receptors have been clinically valuable in treating a wide range of behavioral disorders. The postsynaptic response at a given synapse is determined by the combination of receptor subtypes, G-protein subtypes, and ion channels that are expressed in the postsynaptic cell. Because each of these features can vary both within and among neurons, a tremendous diversity of transmitter-mediated effects is possible. Drugs that influence transmitter actions have enormous importance in the treatment of neurological and psychiatric disorders, as well as in a broad spectrum of other medical problems.

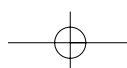
Additional Reading

Reviews

- BARNES, N. M. AND T. SHARP (1999) A review of central 5-HT receptors and their function. *Neuropharm.* 38: 1083–1152.
- BOURIN, M., G. B. BAKER AND J. BRADWEJN (1998) Neurobiology of panic disorder. *J. Psychosomatic Res.* 44: 163–180.
- BURNSTOCK, G. (1999) Current status of purinergic signalling in the nervous system. *Prog. Brain Res.* 120: 3–10.
- CARLSSON, A. (1987) Perspectives on the discovery of central monoaminergic neurotransmission. *Annu. Rev. Neurosci.* 10: 19–40.
- CHANGEUX, J.-P. (1993) Chemical signaling in the brain. *Sci. Amer.* 269 (May): 58–62.
- CIVELLI, O. (1998) Functional genomics: The search for novel neurotransmitters and neuropeptides. *FEBS Letters* 430: 55–58.
- FREDHOLM, B. B. (1995) Adenosine, adenosine receptors and the actions of caffeine. *Pharmacol. Toxicol.* 76: 93–101.
- FREUND, T. F., I. KATONA AND D. PIOMELLI (2003) Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev.* 83: 1017–1066.
- HÖKFELT, T. D. AND 10 OTHERS (1987) Coexistence of peptides with classical neurotransmitters. *Experientia Suppl.* 56: 154–179.
- HYLAND, K. (1999) Neurochemistry and defects of biogenic amine neurotransmitter metabolism. *J. Inher. Metab. Dis.* 22: 353–363.
- INESTROSSA, N. C. AND A. PERELMAN (1989) Distribution and anchoring of molecular forms of acetylcholinesterase. *Trends Pharmacol. Sci.* 10: 325–329.
- IVERSEN, L. (2003) Cannabis and the brain. *Brain* 126: 1252–1270.
- KOOB, G. F., P. P. SANNA AND F. E. BLOOM (1998) Neuroscience of addiction. *Neuron* 21: 467–476.
- KUPFERMANN, I. (1991) Functional studies of cotransmission. *Physiol. Rev.* 71: 683–732.
- LAUBE, B., G. MAKSAY, R. SCHEMM AND H. BETZ (2002) Modulation of glycine receptor function: A novel approach for therapeutic intervention at inhibitory synapses? *Trends Pharmacol. Sci.* 23: 519–527.
- LOVINGER, D. M. (1999) 5-HT₃ receptors and the neural actions of alcohols: An increasingly exciting topic. *Neurochem. Internat.* 35: 125–30.
- MACKENZIE, A. B., A. SURPRENANT AND R. A. NORTH (1999) Functional and molecular diversity of purinergic ion channel receptors. *Ann. NY Acad. Sci.* 868: 716–729.
- MASSON, J., C. SAGN, M. HAMON AND S. E. MESTIKAWY (1999) Neurotransmitter transporters in the central nervous system. *Pharmacol. Rev.* 51: 439–464.
- MELDRUM, B. AND J. GARTHWAITE (1990) Glutamate neurotoxicity may underlie slowly progressive degenerative diseases such as Huntington's Disease and Alzheimer's Disease. *Trends Pharmacol. Sci.* 11: 379–387.
- NAKANISHI, S. (1992) Molecular diversity of glutamate receptors and implication for brain function. *Science* 258: 597–603.
- PERRY, E., M. WALKER, J. GRACE AND R. PERRY (1999) Acetylcholine in mind: A neurotransmitter correlate of consciousness? *Trends Neurosci.* 22: 273–280.
- PIERCE, K. L., R. T. PREMONT AND R. J. LEFKOWITZ (2002) Seven-transmembrane receptors. *Nature Rev. Mol. Cell Biol.* 3: 639–650.
- SCHWARTZ, J. C., J. M. ARRANG, M. GARBARG, H. POLLARD AND M. RUAT (1991) Histaminergic transmission in the mammalian brain. *Physiol. Rev.* 71: 1–51.
- SCHWARTZ, M. W., S. C. WOODS, D. PORTE JR., R. J. SEELEY AND D. G. BASKIN (2000) Central nervous system control of food intake. *Nature* 404: 661–671.
- STAMLER, J. S., E. J. TOONE, S. A. LIPTON AND N. J. SUCHER (1997) (S)NO Signals: Translocation, regulation, and a consensus motif. *Neuron* 18: 691–696.
- TUCEK, S., J. RICNY AND V. DOLEZAL (1990) Advances in the biology of cholinergic neurons. *Adv. Neurol.* 51: 109–115.
- WEBB, T. E. AND E. A. BARNARD (1999) Molecular biology of P2Y receptors expressed in the nervous system. *Prog. Brain Res.* 120: 23–31.
- WILSON, R. I. AND R. A. NICOLL (2002) Endocannabinoid signaling in the brain. *Science* 296: 678–682.

Important Original Papers

- BRENOWITZ, S. D. AND W. G. REGEHR (2003) Calcium dependence of retrograde inhibition by endocannabinoids at synapses onto Purkinje cells. *J. Neurosci.* 23: 6373–6384.
- CHAVAS, J. AND A. MARTY (2003) Coexistence of excitatory and inhibitory GABA synapses in the cerebellar interneuron network. *J. Neurosci.* 23: 2019–2031.
- CHEN, Z. P., A. LEVY AND S. L. LIGHTMAN (1995) Nucleotides as extracellular signalling molecules. *J. Neuroendocrinol.* 7: 83–96.
- CURTIS, D. R., J. W. PHILLIS AND J. C. WATKINS (1959) Chemical excitation of spinal neurons. *Nature* 183: 611–612.
- DALE, H. H., W. FELDBERG AND M. VOGT (1936) Release of acetylcholine at voluntary motor nerve endings. *J. Physiol.* 86: 353–380.
- DAVIES, P. A. AND 6 OTHERS (1999) The 5-HT_{3B} subunit is a major determinant of serotonin-receptor function. *Nature* 397: 359–363.
- GOMEZA, J. AND 6 OTHERS (2003) Inactivation of the glycine transporter 1 gene discloses vital role of glial glycine uptake in glycinergic inhibition. *Neuron* 40: 785–796.
- GU, J. G. AND A. B. MACDERMOTT (1997) Activation of ATP P2X receptors elicits glutamate release from sensory neuron synapses. *Nature* 389: 749–753.
- HÖKFELT, T., O. JOHANSSON, A. LJUNGDAHL, J.



- M. LUNDBERG AND M. SCHULTZBERG (1980) Peptidergic neurons. *Nature* 284: 515–521.
- HOLLMANN, M., C. MARON AND S. HEINEMANN (1994) N-glycosylation site tagging suggests a three transmembrane domain topology for the glutamate receptor GluR1. *Neuron* 13: 1331–1343.
- HUGHES, J., T. W. SMITH, H. W. KOSTERLITZ, L. A. FOTHERGILL, B. A. MORGAN AND H. R. MORRIS (1975) Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* 258: 577–580.
- KAUPMANN, K. AND 10 OTHERS (1997) Expression cloning of GABA β receptors uncovers similarity to metabotropic glutamate receptors. *Nature* 386: 239–246.
- KREITZER, A. C. AND W. G. REGEHR (2001) Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron* 29: 717–727.
- LEDEBT, C. AND 9 OTHERS (1997) Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor. *Nature* 388: 674–678.
- NAVEILHAN, P. AND 10 OTHERS (1999) Normal feeding behavior, body weight and leptin response require the neuropeptide Y Y2 receptor. *Nature Med.* 5: 1188–1193.
- OHNO-SHOSAKU, T., T. MAEJIMA, AND M. KANO (2001) Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* 29: 729–738.
- ROSENMUND, C., Y. STERN-BACH AND C. F. STEVENS (1998) The tetrameric structure of a glutamate receptor channel. *Science*: 280: 1596–1599.
- THOMAS, S. A. AND R. D. PALMITER (1995) Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. *Nature* 374: 640–643.
- UNWIN, N. (1995) Acetylcholine receptor channels imaged in the open state. *Nature* 373: 37–43.
- WANG, Y.M. AND 8 OTHERS (1997) Knockout of the vesicular monoamine transporter 2 gene results in neonatal death and supersensitivity to cocaine and amphetamine. *Neuron* 19: 1285–1296.

Books

- BRADFORD, H. F. (1986) *Chemical Neurobiology*. New York: W. H. Freeman.
- COOPER, J. R., F. E. BLOOM AND R. H. ROTH (2003) *The Biochemical Basis of Neuropharmacology*. New York: Oxford University Press.
- FELDMAN, R. S., J. S. MEYER AND L. F. QUENZER (1997) *Principles of Neuropharmacology*, 2nd Edition. Sunderland, MA: Sinauer Associates.
- HALL, Z. (1992) *An Introduction to Molecular Neurobiology*. Sunderland, MA: Sinauer Associates.
- HILLE, B. (2002) *Ion Channels of Excitable Membranes*, 3rd Edition. Sunderland, MA: Sinauer Associates.
- MYCEK, M. J., R. A. HARVEY AND P. C. CHAMPE (2000) *Pharmacology*, 2nd Edition. Philadelphia, New York: Lippincott/Williams and Wilkins Publishers.
- NICHOLLS, D. G. (1994) *Proteins, Transmitters, and Synapses*. Boston: Blackwell Scientific.
- SIEGEL, G.J., B. W. AGRANOFF, R. W. ALBERS, S. K. FISHER AND M. D. UHLER (1999) *Basic Neurochemistry*. Philadelphia: Lippincott-Raven.

