## Box 16–2 Detection of Chemical Messengers and Their Processing Enzymes Within Neurons

Powerful histochemical techniques are available for detecting both small-molecule transmitter substances and neuroactive peptides in histological sections of nervous tissue.

Catecholamines and serotonin, when reacted with formaldehyde vapor, form fluorescent derivatives. In an early example of transmitter histochemistry, the Swedish neuroanatomists Bengt Falck and Nils Hillarp found that the reaction can be used to locate transmitters with fluorescence microscopy under properly controlled conditions.

Because individual vesicles are too small to be resolved by the light microscope, the exact position of the vesicles containing the transmitter was inferred by comparing the fluorescence under the light microscope with the position of vesicles under the electron microscope. A number of fluorescent false transmitters, particularly those that mimic catecholamines, are substrates for plasma membrane and/or vesicular transporters, enabling their use to label vesicles and assess their turnover in living tissue. In addition, a variety of genetically expressed neurotransmitter reporters based on green fluorescent protein can be used to detect extracellular levels of neurotransmitters.

Histochemical analysis can be extended to the ultrastructure of neurons under special conditions. Fixation of nervous tissue in the presence of potassium permanganate, chromate, or silver salts, or the dopamine analog

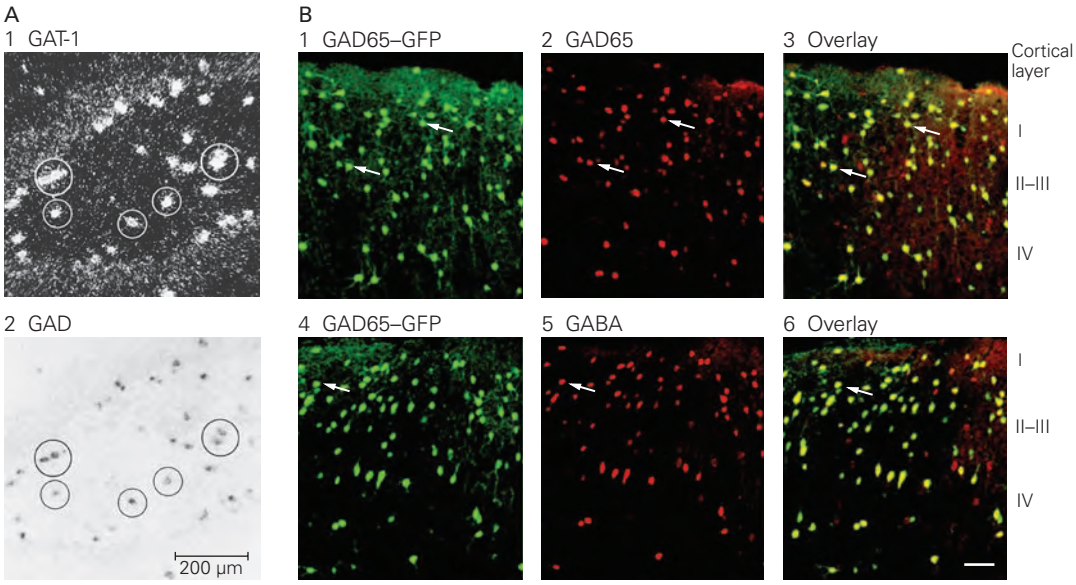


**Figure 16–3** Techniques for visualizing chemical messengers.

**A.** A light-microscope section of the hippocampus of a rat. **1.** In situ hybridization using a probe for the mRNA encoding GAT-1, a GABA transporter. The probe was end-labeled with $\alpha$-$^{35}$S-dATP and visualized by clusters of silver grains in the overlying autoradiographic photographic emulsion. **2.** In situ hybridization of the mRNA for glutamic acid decarboxylase (**GAD**), the specific biosynthetic enzyme for GABA, was carried out with an oligonucleotide probe linked to the enzyme alkaline phosphatase. The GAD probe was visualized by accumulation of colored alkaline phosphatase reaction product in the cytoplasm. Neurons expressing both GAT-1 and GAD transcripts were labeled by silver grains and the phosphatase reaction, respectively, and are indicated by circles enclosing cells bodies that contain both labels. (Used with permission of Sarah Augood.)

**B.** Images of neocortex from a GAD65-GFP transgenic mouse in which green fluorescent protein (**GFP**) is expressed under the control of the GAD65 promotor. GFP is co-localized with GAD65 (1–3) and GABA (4–6) (both detected by indirect immunofluorescence) in neurons in the different layers. Most of the GFP-positive neurons are immunopositive for GAD65 and GABA (**arrows** show selected examples). Scale bar = 100 µm. (Adapted, with permission, from López-Bendito et al. 2004. Copyright © 2004 Oxford University Press.)

5-hydroxdopamine, which forms an electron-dense product, intensifies the electron density of vesicles containing biogenic amines and thus reveals the large number of dense-core vesicles that are characteristic of aminergic neurons.

It is also possible to identify neurons that express the gene for a particular transmitter enzyme or peptide precursor. Many methods for detecting specific mRNAs depend on nucleic acid hybridization. One such method is in situ hybridization.

Two single strands of a nucleic acid polymer will pair if their sequence of bases is complementary. With in situ hybridization, the strand of noncoding DNA (the negative or antisense strand or its corresponding RNA) is applied to tissue sections under conditions suitable for hybridizing with endogenous (sense) mRNA. If the probes are radiolabeled, autoradiography reveals the locations of neurons that contain the complex formed by the labeled complementary nucleic acid strand and the mRNA.

Hybrid oligonucleotides synthesized with nucleotides containing base analogs tagged chemically, fluorescently, or with antibodies can be detected histochemically. Multiple labels can be used at the same time (Figure 16–3A). RNAscope, a more recent mRNA hybridization method, allows for simultaneous detection of different mRNAs with lower background and single-molecule sensitivity. Another approach to detecting the synthetic proteins involves viral or transgenic expression of proteins fused to variants of green fluorescent protein (Figure 16–3B).

Transmitter substances can also be detected using immunohistochemical techniques. Amino acid transmitters, biogenic amines, and neuropeptides have a primary amino group that becomes covalently fixed within the neurons; this group becomes cross-linked to proteins by aldehydes, the usual fixatives used in microscopy for immunohistochemical techniques.

Specific antibodies against the transmitter substances are necessary. Antibodies specific to serotonin, histamine, and many neuroactive peptides can be detected by a second antibody (in a technique called *indirect immunofluorescence*). As an example, if the first antibody is rabbit-derived, the second antibody can be goat antibody raised against rabbit immunoglobulin.

These commercially available secondary antibodies are tagged with fluorescent dyes and used under the fluorescence microscope to locate antigens in regions of individual neurons—cell bodies, axons, and presynaptic release sites (Figure 16–3).

Immunohistochemical techniques are also used with electron microscopy to locate chemical transmitters in the ultrastructure of neurons. Such techniques

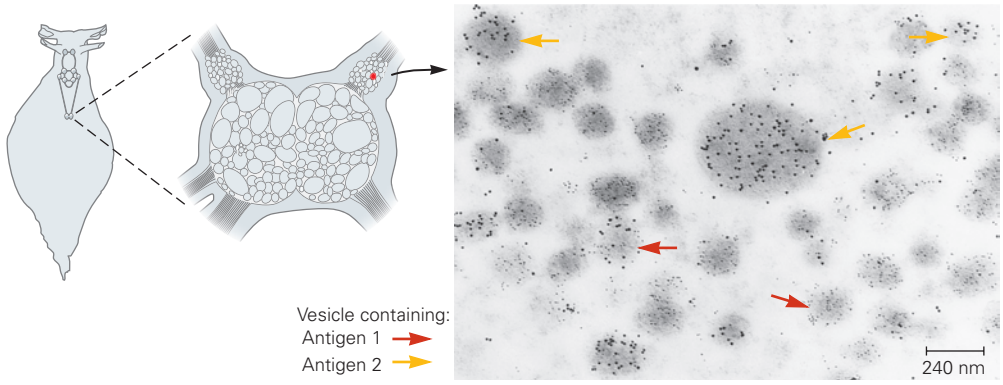


**Figure 16–4** Electron-opaque gold particles linked to antibody are used to locate antigens in tissue at the ultrastructural level. The electron micrograph shows a section through the cell body of an *Aplysia* bag cell. Bag cells control reproductive behavior by releasing a group of neuropeptides cleaved from the egg-laying hormone (ELH) precursor. The cells contain several kinds of dense-core vesicles. The cell shown here was treated with two antibodies against different amino acid sequences contained in different regions of the ELH precursor. One antibody was raised in rabbits and the other in rats. These antibodies were detected with anti-rabbit or anti-rat immunoglobulins (secondary antibodies) raised in goats. Each secondary antibody was coupled to colloidal gold particles of a distinct size. Vesicles identified by antigen 1 (labeled with the smaller gold particles) are smaller than vesicles identified by antigen 2 (labeled with the larger gold particles), indicating that the specific fragments cleaved from the precursor are localized in different vesicles. (Reproduced, with permission, from Fisher et al. 1988.)

(*continued*)

## Box 16–2 Detection of Chemical Messengers and Their Processing Enzymes Within Neurons (continued)

usually involve a peroxidase-antiperoxidase system that produces an electron-dense reaction product. Another method is to use antibodies linked to electron-dense gold particles. Spheres of colloidal gold can be generated with precise diameters in the nanometer range. When coated with an appropriate antibody, these gold particles can be used to detect proteins and peptides with high resolution. This technique has the additional useful feature that more than one specific antibody can be examined in the same tissue section if each antibody is linked to gold particles of different sizes (Figure 16–4).

A number of fluorescent vesicular transporter substrates have been used as fluorescent false neurotransmitters (FFNs) to monitor transmitter release in mouse brain slice or whole fly brain (Figure 16–5). This approach allows visualization of nerve terminals in which synaptic vesicles have been loaded with FFNs; release can then be monitored optically in real time in response to either depolarization, which leads to exocytosis and synaptic vesicle emptying, or amphetamine, which leads to nonexocytic release of vesicular contents into the cytoplasm in response to vesicle deacidification.

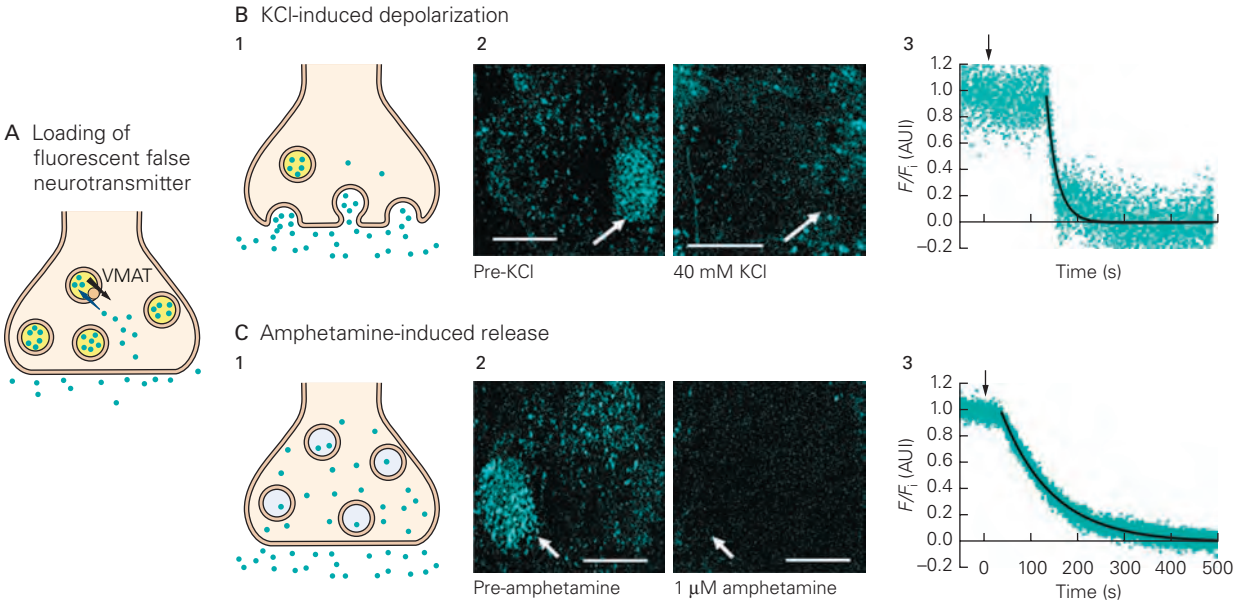


**Figure 16–5** Fluorescent false neurotransmitter (FFN) labeling permits optical monitoring of neurotransmitter release.

**A.** FFN (**blue dots**) is transported by VMAT into synaptic vesicles in dopamine nerve terminals. Vesicles at steady state are acidic, as indicated by **yellow shading**.

**B. 1.** Raising the extracellular KCl concentration leads to depolarization and release of vesicles through exocytosis, resulting in the loss of fluorescent label (destaining). **2.** KCl (40 mM) depolarization caused rapid FFN206 destaining in presynaptic dopamine nerve terminals. Whole fly brains were loaded to steady state with FFN206 (300 nM) and treated with KCl. Projected image stacks of the neuropil before (*left*) and after (*right*) KCl-induced depolarization. **3.** Kinetics of fluorescence decay from representative experiments. **Black arrow** indicates initiation of KCl addition. Scale bar = 25 µm.

**C. 1.** Amphetamine leads to deacidification of vesicles (**loss of yellow shading**) and their destaining through a nonexocytic mechanism discussed in the text. **2.** Amphetamine (1 µM) caused FFN206 destaining. Whole fly brains were loaded to steady state with FFN206 (300 nM) and treated with amphetamine. Projected image stacks of neuropil before (*left*) and after (*right*) treatment. **3.** Kinetics of fluorescence decay from representative experiments. **Black arrow** indicates initiation of drug addition. Scale bar = 25 µm. (Reproduced, with permission, from Freyberg et al. 2016.)

butyrylcholinesterase, which can also degrade other molecules including cocaine and the paralytic drug succinylcholine. However, the precise functions of butyrylcholinesterase are not fully understood.

Many other enzymatic pathways that degrade released transmitters are not directly involved in terminating synaptic transmission but are important for controlling the concentration of the transmitter within the neuron or for inactivating transmitter molecules that have diffused away from the synaptic cleft. Many of these degradative enzymes are important clinically—they provide sites for drug action and serve as diagnostic indicators. For example, inhibitors of MAO, an intracellular enzyme that degrades amine transmitters, are used to treat depression and Parkinson disease. Catechol-*O*-methyltransferase (COMT) is another cytoplasmic enzyme that is important for degrading biogenic amines. Measurement of its metabolites provides a useful clinical index of the efficacy of drugs that affect the synthesis or degradation of the biogenic amines in nervous tissue. COMT is thought to play a particularly critical role in regulating cortical dopamine levels because of the low levels of the dopamine uptake transporter. The relevance of this enzyme is underscored by the finding that a functional polymorphism in the COMT gene has been related to cognitive performance.

Neuropeptides are removed relatively slowly from the synaptic cleft by slow diffusion and proteolysis by extracellular peptidases. In contrast, small-molecule transmitters are removed more quickly from the synaptic cleft and extrasynaptic space. The critical mechanism for inactivation of most small molecule neurotransmitters is reuptake at the plasma membrane. This mechanism serves the dual purposes of terminating the synaptic action of the transmitter as well as recapturing transmitter molecules for subsequent reuse. Although Elliott had hypothesized in 1914 that uptake transporters might exist, their discovery waited until 1958 when F. Barbara Hughes and Benjamin Brodie found that blood platelets accumulated norepinephrine and serotonin, which could compete with each other for uptake. Julius Axelrod,* also a member of Brodie's group, soon afterward characterized norepinephrine uptake into neurons using a radiolabeled substrate.

High-affinity uptake is mediated by transporter molecules in the membranes of nerve terminals and glial cells. Unlike vesicular transporters, which are powered by the $H^+$ electrochemical gradient in an antiport mechanism, plasma membrane transporters are driven by the $Na^+$ electrochemical gradient through a symport mechanism in which $Na^+$ ions and transmitter move in the same direction.

Each type of neuron has its own characteristic uptake mechanism. For example, noncholinergic neurons do not take up choline with high affinity. Certain powerful psychotropic drugs can block uptake processes. For example, cocaine blocks the uptake of dopamine, norepinephrine, and serotonin; the tricyclic antidepressants block uptake of serotonin and norepinephrine. The selective serotonin reuptake inhibitors, such as fluoxetine (Prozac), were an important therapeutic innovation and are generally better tolerated than tricyclic antidepressants, although treatment-resistant depression remains a critical problem. The application of appropriate drugs that block transporters can prolong and enhance synaptic signaling by the biogenic amines and GABA. In some instances, drugs act both on transporters on the neuron's surface and on vesicular transporters within the cell. For example, amphetamines are actively taken up by the dopamine or other biogenic amine transporters on the external membrane of the neuron as well as by VMAT2.

Transporter molecules for neurotransmitters belong to two distinct groups that are different in both structure and mechanism. High-resolution structures of bacterial homologs from each of these families have been solved, which has greatly advanced our understanding of transporter mechanisms.

One group of transporters is the neurotransmitter sodium symporters (NSS), a superfamily of transmembrane proteins that thread through the plasma membrane 12 times (11 times for many prokaryotic homologs). These proteins are comprised of a pseudo-symmetric inverted repeat in which membrane-spanning segments 1 to 5 are homologous to membrane-spanning segments 6 to 10. The NSS family includes the transporters of GABA, glycine, norepinephrine, dopamine, serotonin, osmolytes, and amino acids. Crystal structures for the human serotonin transporter and the fly dopamine transporter, which share the same structure and general mechanism as the bacterial homologs crystallized previously, have recently been solved.

The second family consists of transporters of glutamate. These proteins traverse the plasma membrane eight times and contain two helical hairpins that are

*Axelrod received a bachelor's degree in chemistry and wrote many of his celebrated papers as a technician in Brodie's lab before entering graduate school and receiving a PhD 21 years later. He was awarded a share of the Nobel Prize in 1970 for his co-discovery of neuronal norepinephrine uptake and his discovery of COMT. His co-recipients of the prize that year were Bernard Katz, who described quantal neurotransmission, and Ulf von Euler, who also studied vesicular uptake and epinephrine release.

thought to serve a role in gating access of substrate from each side of the membrane (see Figure 8–16). Each group includes several transporters for each transmitter substance; for example, there are multiple GABA, glycine, and glutamate transporters, each with somewhat different localization, function, and pharmacology.

The two groups can be distinguished functionally. Although both are driven by the electrochemical potential provided by the $Na^+$ gradient, transport of glutamate requires the countertransport of $K^+$, whereas transport by NSS proteins often requires the cotransport of $Cl^-$ (or $H^+$ antiport in the case of prokaryotic homologs). During transport of glutamate, one negatively charged molecule of the transmitter is imported with three $Na^+$ ions and one proton (symport) in exchange for the export of one $K^+$. This leads to a net influx of two positive charges for each transport cycle, generating an inward current. As a result of this charge transfer, the negative resting potential of the cell generates a large inward driving force that results in an enormous gradient of glutamate across the cell membrane. In contrast, the NSS proteins transport one to three $Na^+$ ions and one $Cl^-$ ion together with their substrates. While under most conditions the electrochemical driving force is sufficient for NSS transporters to carry transmitter into the cell, thereby increasing the cytoplasmic transmitter concentration, the concentration of transmitter in the cytoplasm is quite low and ultimately determined by the action of vesicular transporters to load transmitter into synaptic vesicles.

A fascinating aspect of the function of the NSS proteins is the ability of these transporters to run backward, allowing them to generate transmitter efflux. This is best characterized for the neurotransmitter dopamine, as amphetamine and related analogs lead to massive release of dopamine through a nonexocytotic mechanism. As discussed earlier, at pharmacological doses, amphetamine is actively transported by both the plasma membrane dopamine transporter (DAT) and the vesicular VMAT2; the latter effect dissipates the vesicular $H^+$ gradient, leading to the escape of dopamine to the cytoplasm. This dopamine then moves "backward", out of the cell, through DAT, a process that requires phosphorylation of its N-terminus. While essential for amphetamine function, the normal physiological role of this phosphorylation remains a mystery, as it does not seem essential for dopamine uptake. Computational studies suggest that phosphorylation-regulated interactions of the N-terminus with acidic lipids in the inner leaflet play a role in modulating transporter function. Nevertheless, the ultimate answer may require atomic resolution structures that include the N-terminal domain coupled with biophysical data on N-terminal dynamics.

## Highlights

1. Information carried by a neuron is encoded in electrical signals that travel along its axon to a synapse, where these signals are transformed and carried across the synaptic cleft by one or more chemical messengers.
2. Two major classes of chemical messengers, small-molecule transmitters and neuroactive peptides, are packaged in vesicles within the presynaptic neuron. After their synthesis in the cytoplasm, small-molecule transmitters are taken up and highly concentrated in vesicles, where they are protected from degradative enzymes in the cytoplasm.
3. Synaptic vesicles in the periphery are highly concentrated in nerve endings and, in the brain, tend to be at varicosities along the axon at presynaptic sites. Classical excitatory synapses with ionotropic glutamate receptors are examples of "private" synapses that communicate with a closely apposed postsynaptic structure such as a dendritic spine. In contrast, the dopamine system exemplifies "social" synapses that can interact with extrasynaptic receptors on many neurons.
4. To prevent depletion of small molecule transmitters during rapid synaptic transmission, most are synthesized locally at terminals.
5. The protein precursors of neuroactive peptides are synthesized only in the cell body, the site of transcription and translation. The neuropeptides are packaged in secretory granules and vesicles that are carried from the cell body to the terminals by axoplasmic transport. Unlike the vesicles that contain small-molecule transmitters, these vesicles are not refilled at the terminal.
6. The enzymes that regulate transmitter biosynthesis are under tight regulatory control, and changes in neuronal activity can produce homeostatic changes in the levels and activity of these enzymes. This regulation can occur both posttranslationally in the cytoplasm, as a result of phosphorylation and dephosphorylation reactions, as well as by transcriptional control in the nucleus.
7. Precise mechanisms for terminating transmitter actions represent a key step in synaptic

transmission that is nearly as important as transmitter synthesis and release. Some released transmitter is lost as a result of simple diffusion out of the synaptic cleft. However, for the most part, transmitter actions are terminated by specific molecular reactions.

8. Acetylcholine is rapidly hydrolyzed by acetylcholinesterase to choline and acetate. Glutamate, GABA, glycine, and the biogenic amines are taken up into presynaptic terminals and/or glia by specific transporters at the plasma membrane that are driven by the $Na^+$ gradient.
9. Some of the most potent psychoactive compounds act at neurotransmitter transporters. The psychostimulatory effects of cocaine result from its action to prevent reuptake of dopamine, thereby increasing its extracellular levels. In contrast, amphetamine and its derivatives promote nonexocytotic release of dopamine through a mechanism involving both the plasma membrane DAT and the vesicular transporter VMAT2.
10. The first step in understanding the molecular strategy of chemical transmission usually involves identifying the contents of synaptic vesicles. Except for those instances in which transmitter is released by transporter molecules or by diffusion through the membrane (in the case of gases and lipid metabolites, see Chapter 14), only molecules suitably packaged in vesicles can be released from a neuron's terminals. However, not all molecules released by a neuron are chemical messengers—only those that bind to appropriate receptors and initiate functional changes in that target neuron can usefully be considered neurotransmitters.
11. Information is transmitted when transmitter molecules bind to receptor proteins in the membrane of another cell, causing them to change conformation, leading either to increased ion conductance in the case of ligand-gated ion channels or to alterations in downstream signaling pathways in the case of G protein–coupled receptors.
12. The co-release of several neuroactive substances onto appropriate postsynaptic receptors permits great diversity of information to be transferred in a single synaptic action.

Jonathan A. Javitch
David Sulzer

## Selected Reading

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. 2002. Membrane transport of small molecules and the electrical properties of membranes. In: *Molecular Biology of the Cell*, 4th ed. New York and Oxford: Garland Science.

Axelrod J. 2003. Journey of a late blooming biochemical neuroscientist. J Biol Chem 278:1–13.

Burnstock G. 1986. Purines and cotransmitters in adrenergic and cholinergic neurones. Prog Brain Res 68:193–203.

Chaudhry FA, Boulland JL, Jenstad M, Bredahl MK, Edwards RH. 2008. Pharmacology of neurotransmitter transport into secretory vesicles. Handb Exp Pharmacol 184:77–106.

Cooper JR, Bloom FE, Roth RH. 2003. *The Biochemical Basis of Neuropharmacology*, 8th ed. New York: Oxford Univ. Press.

Dale H. 1935. Pharmacology and nerve endings. Proc R Soc Med (Lond) 28:319–332.

Edwards RH. 2007. The neurotransmitter cycle and quantal size. Neuron 55:835–858.

Falck B, Hillarp NÅ, Thieme G, Torp A. 1982. Fluorescence of catecholamines and related compounds condensed with formaldehyde. Brain Res Bull 9:11–15.

Fatt P, Katz B. 1950. Some observations on biological noise. Nature 166:597–598.

Jiang J, Amara SG. 2011. New views of glutamate transporter structure and function: advances and challenges. Neuropharmacology 60:172–181.

Johnson RG. 1988 Accumulation of biological amines into chromaffin granules: a model for hormone and neurotransmitter transport. Physiol Rev 68:232–307.

Katz B. 1966. *Nerve, Muscle, and Synapse*. New York: McGraw-Hill.

Koob GF, Sandman CA, Strand FL (eds). 1990. A decade of neuropeptides: past, present and future. Ann N Y Acad Sci 579:1–281.

Loewi O. 1960. An autobiographic sketch. Perspect Biol Med 4:3–25.

Lohr KM, Masoud ST, Salahpour A, Miller GW. 2017. Membrane transporters as mediators of synaptic dopamine dynamics: implications for disease. Eur J Neurosci 45:20–33.

Pereira D, Sulzer D. 2012. Mechanisms of dopamine quantal size regulation. Front Biosci 17:2740–2767.

Sames D, Dunn M, Karpowicz RJ Jr, Sulzer D. 2013. Visualizing neurotransmitter secretion at individual synapses. ACS Chem Neurosci 4:648–651.

Siegel GJ, Agranoff BW, Albers RW, Molinoff PB (eds). 1998. *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects*, 6th ed. Philadelphia: Lippincott.

Snyder SH, Ferris CD. 2000. Novel neurotransmitters and their neuropsychiatric relevance. Am J Psychiatry 157:1738–1751.

Sossin WS, Fisher JM, Scheller RH. 1989. Cellular and molecular biology of neuropeptide processing and packaging. Neuron 2:1407–1417.

Sulzer D, Cragg SJ, Rice ME. 2016. Striatal dopamine neurotransmission: regulation of release and uptake. Basal Ganglia 6:123–148.

Sulzer D, Pothos EN. 2000. Regulation of quantal size by presynaptic mechanisms. Rev Neurosci 11:159–212.
Toei M, Saum R, Forgac M. 2010. Regulation and isoform function of the V-ATPases. Biochemistry 49:4715–4723.
Torres GE, Amara SG. 2007. Glutamate and monoamine transporters: new visions of form and function. Curr Opin Neurobiol 17:304–312.
Van der Kloot W. 1991 The regulation of quantal size. Prog Neurobiol 36:93–130.
Weihe E, Eiden LE. 2000. Chemical neuroanatomy of the vesicular amine transporters. FASEB J 15:2435–2449.

## References

Augood SJ, Herbison AE, Emson PC. 1995. Localization of GAT-1 GABA transporter mRNA in rat striatum: cellular coexpression with $GAD_{67}$ mRNA, $GAD_{67}$ immunoreactivity, and parvalbumin mRNA. J Neurosci 15:865–874.
Coleman JA, Green EM, Gouaux E. 2016. X-ray structures and mechanism of the human serotonin transporter. Nature 532:334–339.
Danbolt NC, Chaudhry FA, Dehnes Y, et al. 1998. Properties and localization of glutamate transporters. Prog Brain Res 116:23–43.
Fisher JM, Sossin W, Newcomb R, Scheller RH. 1988. Multiple neuropeptides derived from a common precursor are differentially packaged and transported. Cell 54:813–822.
Freyberg Z, Sonders MS, Aguilar JI, et al. 2016. Mechanisms of amphetamine action illuminated through in vivo optical monitoring of dopamine synaptic vesicles. Nat Commun 7:10652.
Hnasko TS, Chuhma N, Zhang H, et al. 2010. Vesicular glutamate transport promotes dopamine storage and glutamate corelease in vivo. Neuron 65:643–656.
Khoshbouei, H, Sen N, Guptaroy B, et al. 2004. N-terminal phosphorylation of the dopamine transporter is required for amphetamine-induced efflux. PLoS Biol 2(3): E78.
Krieger DT. 1983. Brain peptides: what, where, and why? Science 222:975–985.
Liu Y, Kranz DE, Waites C, Edwards RH. 1999. Membrane trafficking of neurotransmitter transporters in the regulation of synaptic transmission. Trends Cell Biol 9:356–363.
Lloyd PE, Frankfurt M, Stevens P, Kupfermann I, Weiss KR. 1987. Biochemical and immunocytological localization of the neuropeptides FMRFamide SCPA, SCPB, to neurons involved in the regulation of feeding in *Aplysia*. J Neurosci 7:1123–1132.
López-Bendito G, Sturgess K, Erdélyi F, Szabó G, Molnár Z, Paulsen O. 2004. Preferential origin and layer destination of GAD65-GFP cortical interneurons. Cereb Cortex 14:1122–1133.
Okuda T, Haga T. 2003. High-affinity choline transporter. Neurochem Res 28:483–488.
Otsuka M, Kravitz EA, Potter DD. 1967. Physiological and chemical architecture of a lobster ganglion with particular reference to γ-aminobutyrate and glutamate. J Neurophysiol 30:725–752.
Palay SL. 1956. Synapses in the central nervous system. J Biophys Biochem Cytol 2:193–202.
Pereira DB, Schmitz Y, Mészáros J, et al. 2016. Fluorescent false neurotransmitter reveals functionally silent dopamine vesicle clusters in the striatum. Nat Neurosci 19:578–586.
Rubin RP. 2007. A brief history of great discoveries in pharmacology: in celebration of the centennial anniversary of the founding of the American Society of Pharmacology and Experimental Therapeutics. Pharmacol Rev 59:289–359.
Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E. 2005. Crystal structure of a bacterial homologue of $Na^+$/$Cl^-$-dependent neurotransmitter transporters. Nature 437:205–223.
Yernool D, Boudker O, Jin Y, Gouaux E. 2004. Structure of a glutamate transporter homologue from *Pyrococcus horikoshii*. Nature 431:811–818.

# Part IV


LVX VENIT IN MVNDVN ET DILEXERVNT HOMINES MAGIS TENEBRAS QVAM LVCEM. Io.3.19.
ANTRVM PLATONICVM

*Preceding Page*

In Plato's "Allegory of the Cave," which addresses the origin of knowledge, his early insight into the constructive nature of perception offers illuminating metaphors for the process. The parable begins with the premise that a group of prisoners has never seen the outside world. Their experience is limited to shadows cast upon the wall of the cave by objects passing before a fire. The causes of those shadows—even the fact that they are shadows—is unknown to the prisoners. Nonetheless, over time, the shadows become imbued with meaning in the prisoners' minds. Metaphorically, the shadows represent sensations, which are fleeting and incoherent. The assignment of meaning represents the construction of intelligible percepts. The prisoner turning the corner of the wall has been freed to witness the larger world of causes, which he reports back to those still imprisoned. In a novel metaphorical take on this ancient story, this returning prisoner represents the field of modern neuroscience, which sheds light on the relationship between our shadowy sensations and our rich perceptual experience of the world. (Plato's Cave, 1604. Jan Pietersz Saenredam, after Cornelis Cornelisz van Haarlem. National Gallery, Washington D.C.)