channel, comprised of four repeats of a domain with six membrane-spanning segments that includes the S4 voltage-sensor and pore-lining P-region (see Figure 8–10). Calcium channels also have auxiliary subunits (termed α_2, β, γ, and δ) that modify the properties of the channel formed by the α_1-subunit. The subcellular localization in neurons of different types of calcium channels also varies. The N- and P/Q-type Ca^{2+} channels are found predominantly in the presynaptic terminal, whereas L-, R-, and T-type channels are found largely in the soma and dendrites.

Four of the types of voltage-gated Ca^{2+} channels—the L-type, P/Q-type, N-type, and R-type—generally require fairly strong depolarization to be activated (voltages positive to −40 to −20 mV are required) and thus are sometimes loosely referred to as *high-voltage-activated* Ca^{2+} channels (Table 15–1). In contrast, T-type channels open in response to small depolarizations around the threshold for generating an action potential (−60 to −40 mV) and are therefore called *low-voltage-activated* Ca^{2+} channels. Because they are activated by small changes in membrane potential, the T-type channels help control excitability at the resting potential and are an important source of the excitatory current that drives the rhythmic pacemaker activity of certain cells in both the brain and heart.

In neurons, the rapid release of conventional transmitters during fast synaptic transmission is mediated mainly by P/Q-type and N-type Ca^{2+} channels, the channel types most concentrated at the active zone. The localization of N-type Ca^{2+} channels at the frog neuromuscular junction has been visualized using a fluorescence-labeled snail toxin that binds selectively to these channels (see Figure 15–4A). The L-type channels are not found in the active zone and thus do not normally contribute to the fast release of conventional transmitters such as ACh and glutamate. However, Ca^{2+} influx through L-type channels is important for slower forms of release that do not occur at specialized active zones, such as the release of neuropeptides from neurons and of hormones from endocrine cells. As we shall see later, regulation of Ca^{2+} influx into presynaptic terminals controls the amount of transmitter release and hence the strength of synaptic transmission.

Mutations in voltage-gated Ca^{2+} channels are responsible for certain acquired and genetic diseases. Timothy syndrome, a developmental disorder characterized by a severe form of autism with impaired cognitive function and a range of other pathophysiological changes, results from a mutation in the α_1-subunit of L-type channels that alters their voltage-dependent gating, thereby affecting dendritic integration. Different point mutations in the P/Q-type channel α_1-subunit give rise to hemiplegic migraine or epilepsy. Patients with Lambert-Eaton syndrome, an autoimmune disease associated with muscle weakness, make antibodies to the P/Q-type channel α_1-subunit that decrease total Ca^{2+} current (Chapter 57).

Transmitter Is Released in Quantal Units

How does the influx of Ca^{2+} trigger transmitter release? Katz and his colleagues provided a key insight into this question by showing that transmitter is released in discrete amounts they called *quanta.* Each quantum of transmitter produces a postsynaptic potential of fixed size, called the *quantal synaptic potential.* The total postsynaptic potential is made up of a large number of quantal potentials. EPSPs seem smoothly graded in amplitude only because each quantal (or unit) potential is small relative to the total potential.

Katz and Fatt obtained the first clue as to the quantal nature of synaptic transmission in 1951 when they observed spontaneous postsynaptic potentials of approximately 0.5 mV at the nerve-muscle synapse of the frog. Like end-plate potentials evoked by nerve stimulation, these small depolarizing responses are largest at the site of nerve-muscle contact and decay electrotonically with distance (see Figure 12–5). Small spontaneous potentials have since been observed in mammalian muscle and in central neurons. Because postsynaptic potentials at vertebrate nerve-muscle synapses are called end-plate potentials, Fatt and Katz called these spontaneous potentials *miniature end-plate potentials*.

Several results convinced Fatt and Katz that the miniature end-plate potentials represented responses to the release of small amounts of ACh, the neurotransmitter used at the nerve-muscle synapse. The time course of the miniature end-plate potentials and the effects of various drugs on them are indistinguishable from the properties of the end-plate potential. Like the end-plate potentials, the miniature end-plate potentials are enhanced and prolonged by prostigmine, a drug that blocks hydrolysis of ACh by acetylcholinesterase. Conversely, they are abolished by agents that block the ACh receptor, such as curare. The miniature end-plate potentials represent responses to small packets of transmitter that are spontaneously released from the presynaptic nerve terminal in the absence of an action potential. Their frequency can be increased by a small depolarization of the presynaptic terminal. They disappear if the presynaptic motor nerve degenerates and reappear when a new motor synapse is formed.

What could account for the small, fixed size of the miniature end-plate potential? Del Castillo and Katz first tested the possibility that each event represents a response to the opening of a *single* ACh receptor-channel. However, application of very small amounts of ACh to the frog muscle end-plate elicited depolarizing postsynaptic responses that were much smaller than the 0.5 mV response of a miniature end-plate potential. This finding made it clear that the miniature end-plate potential represents the opening of more than one ACh receptor-channel. In fact, Katz and Miledi were later able to estimate the voltage response to the elementary current through a single ACh receptor-channel as being only approximately 0.3 μV (Chapter 12). Based on this estimate, a miniature end-plate potential of 0.5 mV would represent the summation of the elementary currents of approximately 2,000 channels. Later work showed that a miniature end-plate potential is the response to the synchronous release of approximately 5,000 molecules of ACh.

What is the relationship of the large end-plate potential evoked by nerve stimulation and the small, spontaneous miniature end-plate responses? This question was first addressed by del Castillo and Katz in a study of synaptic signaling at the nerve-muscle synapse bathed in a solution low in Ca^{2+}. Under this condition, the end-plate potential is reduced markedly, from the normal 70 mV to about 0.5 to 2.5 mV. Moreover, the amplitude of each successive end-plate potential now varies randomly from one stimulus to the next; often, no response can be detected at all (termed *failures*). However, the minimum response above zero—the unit end-plate potential in response to a presynaptic action potential—is identical in amplitude (approximately 0.5 mV) and shape to the spontaneous miniature end-plate potentials. Importantly, the amplitude of each end-plate potential is an integral multiple of the unit potential (Figure 15–6).

Now del Castillo and Katz could ask: How does the rise of intracellular Ca^{2+} that accompanies each action potential affect the release of transmitter? They found that increasing the external Ca^{2+} concentration does not change the amplitude of the unit synaptic potential. However, the proportion of failures decreases and the incidence of higher-amplitude responses (composed of multiple quantal units) increases. These observations show that an increase in external Ca^{2+} concentration does not enhance the *size* of a quantum of transmitter (that is, the number of ACh molecules in each quantum) but rather acts to increase the average number of quanta that are released in response to a presynaptic action potential. The greater the Ca^{2+} influx into the terminal, the larger the number of transmitter quanta released.

Thus, three findings led del Castillo and Katz to conclude that transmitter is released in packets with a fixed amount of transmitter, a quantum: The amplitude of the end-plate potential varies in a stepwise manner at low levels of ACh release, the amplitude of each step increase is an integral multiple of the unit potential, and the unit potential has the same mean amplitude and shape as that of the spontaneous miniature end-plate potentials. Moreover, by analyzing the statistical distribution of end-plate potential amplitudes, del Castillo and Katz and other subsequent researchers were able to show that a single action potential produced a transient increase in the probability that a given quantum of transmitter is released according to a random process, similar to that governing the outcome of a coin toss (Box 15–1).

In the absence of an action potential, the rate of quantal release is low—only one quantum per second is released spontaneously at the end-plate. In contrast, the firing of an action potential releases approximately 150 quanta, each approximately 0.5 mV in amplitude, resulting in a large end-plate potential. Thus, the influx of Ca^{2+} into the presynaptic terminal during an action potential dramatically increases the rate of quantal release by a factor of 150,000, triggering the synchronous release of about 150 quanta in about 1 ms.

Transmitter Is Stored and Released by Synaptic Vesicles

What morphological features of the cell might account for the quantal release of transmitter? The physiological observations indicating that transmitter is released in fixed quanta coincided with the discovery, through electron microscopy, of accumulations of small clear vesicles in the presynaptic terminal. Del Castillo and Katz speculated that the vesicles were organelles for the storage of transmitter, each vesicle stored one quantum of transmitter (amounting to several thousand molecules), and each vesicle released its entire contents into the synaptic cleft in an all-or-none manner at sites specialized for release.

The sites of release, the active zones, contain a cloud of synaptic vesicles that cluster above a fuzzy electron-dense material attached to the internal face of the presynaptic membrane (see Figure 15–4A). At all synapses, the vesicles are typically clear, small, and ovoid, with a diameter of approximately 40 nm (in distinction with the large dense-core vesicles described in Chapter 16). Although most synaptic vesicles do not contact the active zone, some are physically bound. These are called the *docked* vesicles and are thought to

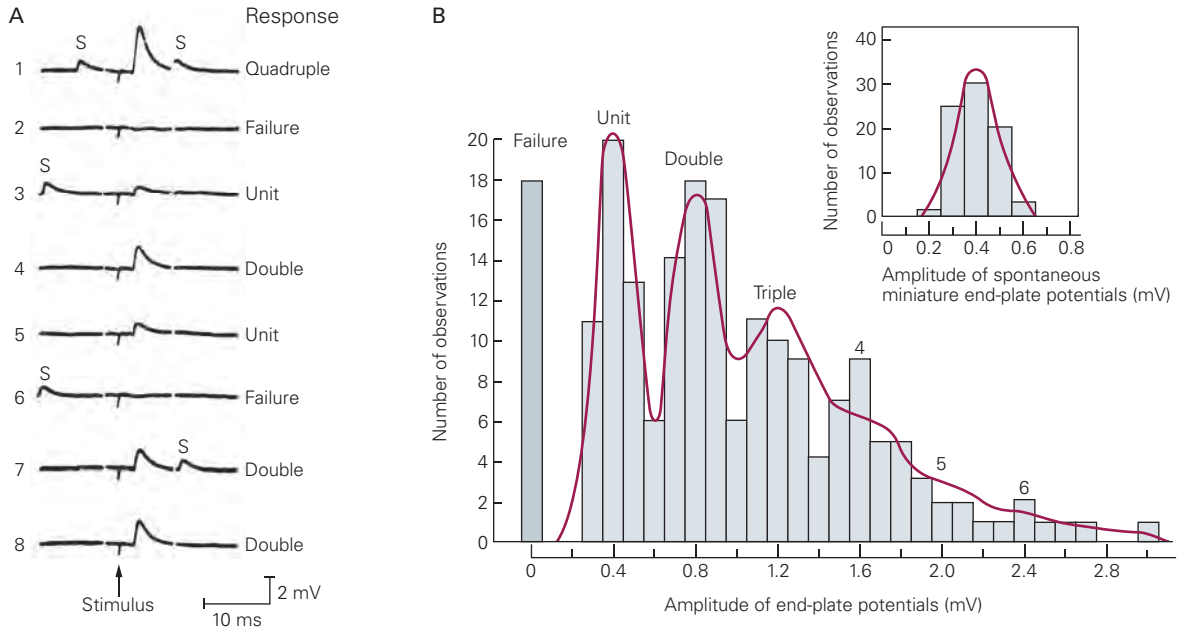


Figure 15–6 **Neurotransmitter is released in fixed increments. Each increment or quantum of transmitter produces a unit end-plate potential of fixed amplitude.** The amplitude of the response evoked by nerve stimulation is thus equal to the amplitude of the unit end-plate potential multiplied by the number of quanta of transmitter released.

A. Intracellular recordings from a muscle fiber at the end-plate show the change in postsynaptic potential when eight consecutive stimuli of the same size are applied to the motor nerve. To reduce transmitter release and to keep the end-plate potentials small, the tissue is bathed in a Ca^{2+}-deficient (and magnesium-rich) solution. The postsynaptic responses to the nerve stimulus vary. Two of the eight presynaptic stimuli elicit no EPSP (failures), two produce unit potentials, and the others produce EPSPs that are approximately two to four times the amplitude of the unit potential. Note that the spontaneous miniature end-plate potentials (**S**), which occur at random intervals in the traces, are the same size as the unit potential. (Adapted, with permission, from Liley 1956.)

B. After many end-plate potentials are recorded, the number of responses with a given amplitude is plotted as a function of this amplitude in the histogram shown here. The distribution of responses falls into a number of peaks. The first peak, at 0 mV, represents failures. The first peak of responses, at 0.4 mV, represents the unit potential, the smallest elicited response. The unit response has the same amplitude as the spontaneous miniature end-plate potentials (inset), indicating that the unit response is caused by the release of a single quantum of transmitter. The other peaks in the histogram are integral multiples of the amplitude of the unit potential; that is, responses are composed of two, three, four, or more quantal events.

The number of responses under each peak divided by the total number of events in the entire histogram is the probability that a single presynaptic action potential triggers the release of the number of quanta that comprise the peak. For example, if there are 30 events in the peak corresponding to the release of two quanta out of a total of 100 events recorded, the probability that a presynaptic action potential releases exactly two quanta is 30/100 or 0.3. This probability follows a Poisson distribution (**red curve**). This theoretical distribution is composed of the sum of several Gaussian functions. The spread of the unit peak (standard deviation of the Gaussian function) reflects the fact that the amount of transmitter in a quantum, and hence the amplitude of the quantal postsynaptic response, varies randomly about a mean value. The successive Gaussian peaks widen progressively because the variability (or variance) associated with each quantal event increases linearly with the number of quanta per event. The distribution of amplitudes of the spontaneous miniature potentials (inset) is fit by a Gaussian curve whose width is identical to that of the Gaussian curve for the unit synaptic responses. (Adapted, with permission, from Boyd and Martin 1956.)

be the ones immediately available for release (sometimes referred to as the *readily releasable pool*). At the neuromuscular junction, the active zones are linear structures (see Figure 15–4), whereas in central synapses, they are disc-shaped structures approximately 0.1 μm^2 in area with dense projections pointing into the cytoplasm. Active zones are generally found in precise apposition to the postsynaptic membrane patches that contain the neurotransmitter receptors (see Figure 13–2). Thus, presynaptic and postsynaptic specializations are functionally and morphologically attuned to each other, sometimes precisely aligned in structural "nanocolumns." As we shall learn later, several key active zone proteins involved in transmitter release have now been identified and characterized.

Box 15–1 Synaptic Strength Depends on the Probability of Transmitter Release and Other Quantal Parameters

The mean size of a synaptic response E evoked by an action potential has often been described as the product of the total number of releasable quanta (n), the probability that an individual quantum of transmitter is released (p), and the size of the response to a quantum (a):

$$E = n \cdot p \cdot a.$$

These parameters are statistical terms, useful for describing the size and variability of the postsynaptic response. At some but not all central synapses, they can also be assigned to biological processes. We begin by focusing on synapses of the kind envisioned by Katz and colleagues, where the interpretation of the parameters is most straightforward. At these synapses, the presynaptic terminal typically contains multiple active zones, and each active zone releases at most a single vesicle in response to an action potential (*univesicular release*).

We then consider another kind of synapse that requires a different interpretation. At these synapses, each active zone can release multiple vesicles in response to a single action potential (*multivesicular release*), leading to very high concentrations of transmitter in the synaptic cleft that can cause the postsynaptic receptors to become saturated with transmitter.

Univesicular Release at Multiple Active Zones

In the simplest case, the parameter a is the response of the postsynaptic membrane to the release of a single vesicle's contents of transmitter. It is assumed that transmitter is packaged in synaptic vesicles, that release of the contents of a vesicle is a stereotyped, all-or-none event, and that single release events occur in physical isolation from each other. Quantal size depends on the amount of transmitter in a vesicle and on the properties of the postsynaptic cell, such as the membrane resistance and capacitance (which can be independently estimated) and the responsiveness of the postsynaptic membrane to the transmitter substance. This can also be measured experimentally by the postsynaptic membrane's response to the application of a known amount of transmitter.

The parameter n describes the maximum number of quantal units that can be released in response to a single action potential if the probability p reaches 1.0. At some central synapses, this maximum may be imposed by the number of release sites (active zones) in the terminals of a presynaptic neuron that contact a given postsynaptic neuron. Multiple studies have found that for this kind of connection n corresponds with the number of release sites determined by electron microscopy, as if those sites obeyed a rough rule wherein a presynaptic action potential triggers the exocytosis of at most one vesicle per active zone.

The parameter p represents the likelihood of vesicle release. This likelihood encompasses a series of events necessary for a particular release site to contribute a quantal event: (1) The active zone must be loaded with at least one releasable vesicle (a process referred to as vesicle mobilization); (2) the presynaptic action potential must evoke Ca^{2+} influx in sufficient quantity and proximity to the vesicle; and (3) the Ca^{2+}-sensitive synaptotagmin and SNARE machinery must cause the vesicle to fuse and discharge its contents.

Here, we focus mainly on the determinants of p. We can treat quantal release at a single active zone as a random event with only two possible outcomes in response to an action potential—the quantum of transmitter is or is not released. Because the quantal responses from different active zones are thought to occur independently of each other in some situations, this is similar to tossing a set of n coins in the air and counting the number of heads or tails. The equivalent of individual coin flips (Bernoulli trials) are then totaled up in a binomial distribution, where p stands for the average probability of success (that is, the probability that any given quantum will be released) and q (equal to $1 - p$) stands for the mean probability of failure.

Both the average probability (p) that an individual quantum will be released and the maximal number (n)

(*continued*)

Quantal transmission has been demonstrated at all chemical synapses so far examined. Nevertheless, the efficacy of transmitter release from a single presynaptic cell onto a single postsynaptic cell varies widely in the nervous system and depends on several factors: (1) the number of individual synapses between a pair of presynaptic and postsynaptic cells (that is, the number of presynaptic boutons that contact the postsynaptic cell); (2) the number of active zones in an individual synaptic terminal; and (3) the probability that a presynaptic action potential will trigger release of one or more quanta of transmitter

Box 15–1 Synaptic Strength Depends on the Probability of Transmitter Release and Other Quantal Parameters (continued)

of releasable quanta are assumed to be constant. (Any reduction in the store of vesicles is assumed to be quickly replenished after each stimulus.) The product of n and p yields an estimate m of the mean number of quanta that will be released. This mean is called the *quantal content* or *quantal output.*

Calculation of the probability of transmitter release can be illustrated with the following example. Consider a terminal that has a releasable store of five quanta ($n = 5$). Assuming $p = 0.1$, then the probability that an individual quantum will not be released from the terminals (q) is $1 - p$, or 0.9. We can now determine the probability that a stimulus will release no quanta (failure), a single quantum, or any other number of quanta (up to n).

The probability that none of the five available quanta will be released by a given stimulus is the product of the individual probabilities that each quantum will not be released: $q^5 = (0.9)^5$, or 0.59. We would thus expect to see 59 failures in a hundred stimuli. The probabilities of observing zero, one, two, three, four, or five quanta are represented by the successive terms of the binomial expansion:

$$(q + p)^5 = q^5 \text{ (failures)} + 5\,q^4p \text{ (1 quantum)} + 10\,q^3p^2 \text{ (2 quanta)} + 10\,q^2p^3 \text{ (3 quanta)} + 5\,qp^4 \text{ (4 quanta)} + p^5 \text{ (5 quanta).}$$

Thus, in 100 stimuli, the binomial expansion would predict 33 single unit responses, 7 double responses, 1 triple response, and 0 quadruple or quintuple responses.

Values for the quantal output m vary from approximately 100 to 300 at the vertebrate nerve-muscle synapse, the squid giant synapse, and *Aplysia* central synapses, to as few as 1 to 4 in the synapses of the sympathetic ganglion and spinal cord of vertebrates. The probability of release p also varies, ranging from as high as 0.7 at the neuromuscular junction in the frog and 0.9 in the crab down to around 0.1 at some mammalian central synapses. Estimates for n range from as much as 1,000 (at the vertebrate nerve-muscle synapse) to 1 (at single terminals of mammalian central neurons).

This numerical example illustrates a characteristic feature of synapses with simple binomial features—their substantial variability. This holds just as strongly whether p is high or low. For example, for $p = 0.9$ and 100 stimuli, the binomial expansion predicts 0 failures, 0 single unit responses, 1 double response, 7 triple responses, 33 quadruple responses, and 59 quintuple responses, the mirror-image of the distribution for $p = 0.1$. Even if each sequential event that supports vesicle release is highly likely, the aggregate strength of the synapse will vary widely.

Multivesicular Release with Receptor Saturation

One well-studied mechanism for achieving high synaptic reliability is through the release of multiple vesicles onto a single postsynaptic site. In the extreme, this can release sufficient amounts of transmitter in the synaptic cleft to cause the postsynaptic receptor binding sites to become fully occupied by transmitter (receptor saturation).

Under these conditions, the postsynaptic response will reach a maximal amplitude. Further release of transmitter, for example in response to a modulatory neurotransmitter, would fail to increase the postsynaptic response. Variability in response size would shrink greatly if, say, three to five vesicles worth of transmitter activated the same number of receptors as a single vesicle. The postsynaptic response would be highly stereotyped (it would appear to result from release of a single quantum of transmitter) even though the presynaptic terminal was releasing multiple vesicles. However, the binomial treatment could still retain some usefulness as a way of adding up the contributions of multiple synapses of this kind, so long as each synapse released transmitter simultaneously and independently. But in such a case, n, p, and a would take on biological meanings different from those in which only a single vesicle could be released per synapse.

at an active zone. As we will see later, release probability can be powerfully regulated as a function of neuronal activity.

In the central nervous system, most presynaptic boutons have only a single active zone where an action potential usually releases at most a single quantum of transmitter in an all-or-none manner. However, at some central synapses, such as the calyx of Held, transmitter is released from a large presynaptic terminal that may contain many active zones and thus can release a large number of quanta in response to a single presynaptic action potential. Central neurons also vary in

the number of synapses that a typical presynaptic cell forms with a typical postsynaptic cell. Whereas most central neurons form only a few synapses with any one postsynaptic cell, a single climbing fiber from neurons in the inferior olive forms up to 10,000 terminals on a single Purkinje neuron in the cerebellum! Finally, the mean probability of transmitter release from a single active zone also varies widely among presynaptic terminals, from less than 0.1 (that is, a 10% chance that a presynaptic action potential will trigger release of a vesicle) to greater than 0.9. This wide range of probabilities can even be seen among the boutons at individual synapses between a specific type of presynaptic cell and a specific type of postsynaptic cell.

Thus, central neurons vary widely in the efficacy and reliability of synaptic transmission. Synaptic *reliability* is defined as the probability that an action potential in a presynaptic cell leads to some measurable response in the postsynaptic cell—that is, the probability that a presynaptic action potential will release one or more quanta of transmitter. *Efficacy* refers to the mean amplitude of the synaptic response, which depends on both the reliability of synaptic transmission and on the mean size of the response when synaptic transmission does occur.

Most central neurons communicate at synapses that have a low probability of transmitter release. The high failure rate of release at most central synapses (that is, their low release probability) is not a *design defect* but serves a purpose. As we discuss later, this feature allows transmitter release to be regulated over a wide dynamic range, which is important for adapting neural signaling to different behavioral demands. In synaptic connections where a low probability of release is deleterious for function, this limitation can be overcome by simply having many active zones in one synapse, as is the case at the calyx of Held and the nerve-muscle synapse. Both contain hundreds of independent active zones, so an action potential reliably releases 150 to 250 quanta, ensuring that a presynaptic signal is always followed by a postsynaptic action potential. Reliable transmission at the neuromuscular junction is essential for survival. An animal would not survive if its ability to move away from a predator was hampered by a low-probability response. Another strategy for increasing reliability is to use multivesicular release, the simultaneous fusion of multiple vesicles at a single active zone, to ensure that postsynaptic receptors are consistently exposed to a saturating concentration of neurotransmitter (see Box 15–1).

Not all chemical signaling between neurons depends on the synaptic machinery described earlier. Some substances, such as certain lipid metabolites and the gas nitric oxide (Chapter 14), can diffuse across the lipid bilayer of the membrane. Others can be moved out of nerve endings by carrier proteins if their intracellular concentration is sufficiently high. Plasma membrane transporters for glutamate or GABA normally take up transmitter into a cell from the synaptic cleft following a presynaptic action potential (Chapter 13). However, in some glial cells of the retina, the direction of glutamate transport can be reversed under certain conditions, causing glutamate to leave the cell through the transporter into the synaptic cleft. Still other substances simply leak out of nerve terminals at a low rate. Surprisingly, approximately 90% of the ACh that leaves the presynaptic terminals at the neuromuscular junction does so through continuous leakage. This leakage is ineffective, however, because it is diffuse and not targeted to receptors at the end-plate region and because it is continuous and low level rather than synchronous and concentrated.

Synaptic Vesicles Discharge Transmitter by Exocytosis and Are Recycled by Endocytosis

The quantal hypothesis of del Castillo and Katz has been amply confirmed by direct experimental evidence that synaptic vesicles do indeed package neurotransmitter and that they release their contents by directly fusing with the presynaptic membrane, a process termed *exocytosis*.

Forty years ago, Victor Whittaker discovered that the synaptic vesicles in the motor nerve terminals of the electric organ of the electric fish *Torpedo* contain a high concentration of ACh. Later, Thomas Reese and John Heuser and their colleagues obtained electron micrographs that caught vesicles in the act of exocytosis. To observe the brief exocytotic event, they rapidly froze the nerve-muscle synapse by immersing it in liquid helium at precisely defined intervals after the presynaptic nerve was stimulated. In addition, they increased the number of quanta of transmitter discharged with each nerve impulse by applying the drug 4-aminopyridine, a compound that blocks certain voltage-gated K^+ channels, thus increasing the duration of the action potential and enhancing Ca^{2+} influx. (The spike broadening produced by this pharmacological intervention resembles spike broadening resulting from cumulative inactivation of K^+ channels during repetitive firing; see Figure 15–15C.) In both cases, prolonged action potentials evoke greater opening of presynaptic Ca^{2+} channels.

These techniques provided clear images of synaptic vesicles at the active zone during exocytosis. Using a technique called *freeze-fracture electron microscopy*,

Reese and Heuser noted deformations of the presynaptic membrane along the active zone immediately after synaptic activity, which they interpreted as invaginations of the cell membrane caused by fusion of synaptic vesicles. These deformations lay along one or two rows of unusually large intramembranous particles, visible along both margins of the presynaptic density. Many of these particles are now thought to be voltage-gated Ca^{2+} channels (Figure 15–7). The particle density (approximately 1,500 per μm^2) is similar to the Ca^{2+} channel density that is thought to be present in the presynaptic plasma membrane at the active zone. Moreover, the proximity of the particles to the release site is consistent with the short time interval between the onset of the Ca^{2+} current and the release of transmitter.

Finally, Heuser and Reese found that these deformations are transient; they occur only when vesicles are discharged and do not persist after transmitter has been released. Thin-section electron micrographs revealed a number of omega-shaped (Ω) structures with the appearance of synaptic vesicles that have just fused with the membrane, prior to the complete collapse of the vesicle membrane into the plasma membrane (Figure 15–7B). Heuser and Reese confirmed this idea by showing that the number of Ω-shaped structures is directly correlated with the size of the EPSP when they varied the concentration of 4-aminopyridine to alter the amount of transmitter release. These morphological studies provide striking evidence that transmitter is released from synaptic vesicles by means of exocytosis.

Following exocytosis, the excess membrane added to the presynaptic terminal is retrieved. In images of presynaptic terminals made 10 to 20 seconds after stimulation, Heuser and Reese observed new structures at the plasma membrane, the coated pits, which are formed by the protein *clathrin* that helps mediate membrane retrieval through the process of endocytosis (Figure 15–7C). Several seconds later, the coated pits are seen to pinch off from the membrane and appear as coated vesicles in the cytoplasm. As we will see later, endocytosis through coated pit formation represents one of several means of vesicle membrane retrieval.

Capacitance Measurements Provide Insight Into the Kinetics of Exocytosis and Endocytosis

In certain neurons with large presynaptic terminals, the increase in surface area of the plasma membrane during exocytosis can be detected in electrical measurements as increases in membrane capacitance. As we saw in Chapter 9, the capacitance of the membrane is proportional to its surface area. Erwin Neher discovered that one could use measurements of capacitance to monitor exocytosis in secretory cells.

In adrenal chromaffin cells (which release epinephrine and norepinephrine) and in mast cells of the rat peritoneum (which release histamine and serotonin), individual dense-core vesicles are large enough to permit measurement of the increase in capacitance associated with fusion of a single vesicle. Release of transmitter in these cells is accompanied by stepwise increases in capacitance, followed somewhat later by stepwise decreases, which reflect the retrieval and recycling of the excess membrane (Figure 15–8).

In neurons, the changes in capacitance caused by fusion of single, small synaptic vesicles are usually too small to resolve. In certain favorable synaptic preparations that release large numbers of vesicles (such as the giant presynaptic terminals of bipolar neurons in the retina), membrane depolarization triggers a transient smooth rise and fall in the total capacitance of the terminal as a result of the exocytosis and retrieval of the membrane from hundreds of individual synaptic vesicles (Figure 15–8C). These results provide direct measurements of the rates of membrane fusion and retrieval.

Exocytosis Involves the Formation of a Temporary Fusion Pore

Morphological studies of mast cells using rapid freezing suggest that exocytosis depends on the formation of a temporary fusion pore that spans the membranes of the vesicle and plasma membranes. In electrophysiological studies of capacitance increases in mast cells, a channel-like fusion pore was detected in the electrophysiological recordings prior to complete fusion of vesicles and cell membranes. This fusion pore starts out with a single-channel conductance of approximately 200 pS, similar to that of gap-junction channels, which also bridge two membranes. During exocytosis, the pore rapidly dilates, probably from around 5 to 50 nm in diameter, and the conductance increases dramatically (Figure 15–9A).

The fusion pore is not just an intermediate structure leading to exocytosis of transmitter, as transmitter can be released through the pore prior to pore expansion and vesicle collapse. This was first shown by amperometry, a method that uses an extracellular carbon-fiber electrode to detect certain amine neurotransmitters, such as serotonin, based on an electrochemical reaction between the transmitter and the electrode that generates an electrical current proportional to the local transmitter concentration. Firing of an action potential in serotonergic cells leads to a large transient increase in electrode

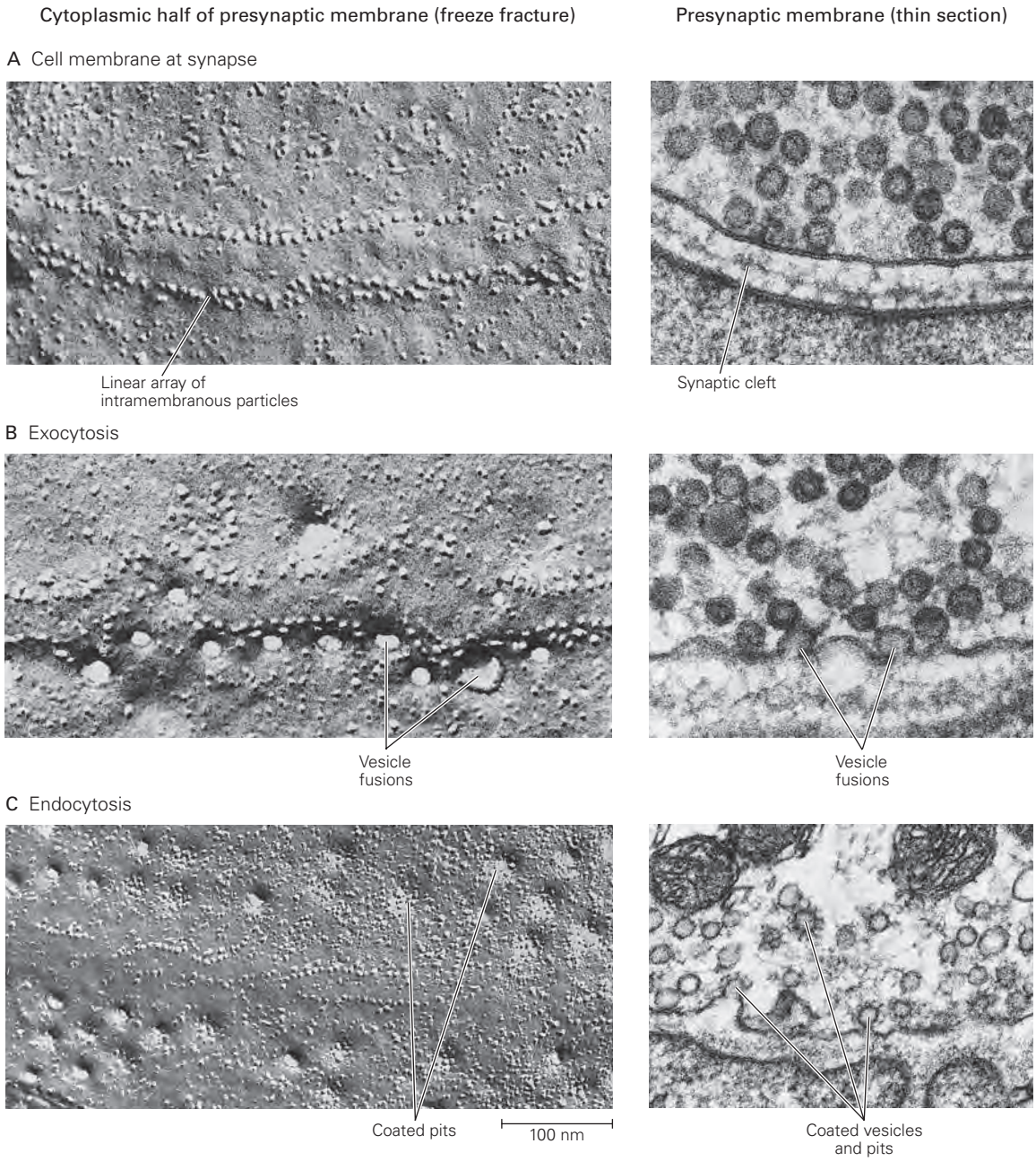


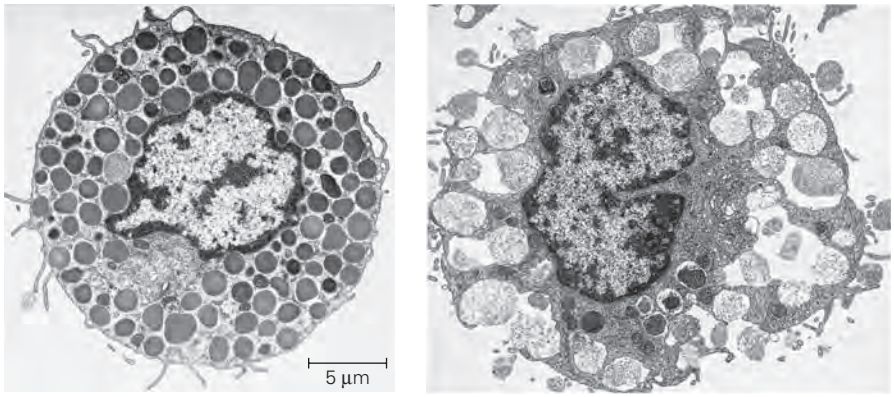
Figure 15–7 **Synaptic vesicles release transmitter by exocytosis and are retrieved by endocytosis.** The images on the left are freeze-fracture electron micrographs at a neuromuscular junction. The freeze-fracture technique exposes the intramembranous area to view by splitting the membrane along the hydrophobic interior of the lipid bilayer. The views shown are of the cytoplasmic leaflet of the bilayer presynaptic membrane looking up from the synaptic cleft (see Figure 15–4A). Conventional thin-section electron micrographs on the right show cross-section views of the presynaptic terminal, synaptic cleft, and postsynaptic muscle membrane. (Reproduced, with permission, from Heuser and Reese 1981. Permission conveyed through Copyright Clearance Center, Inc.)

A. Parallel rows of intramembranous particles arrayed on either side of an active zone are thought to be the voltage-gated Ca^{2+} channels essential for transmitter release (see Figure 15–4A). The thin-section image at right shows the synaptic vesicles adjacent to the active zone.

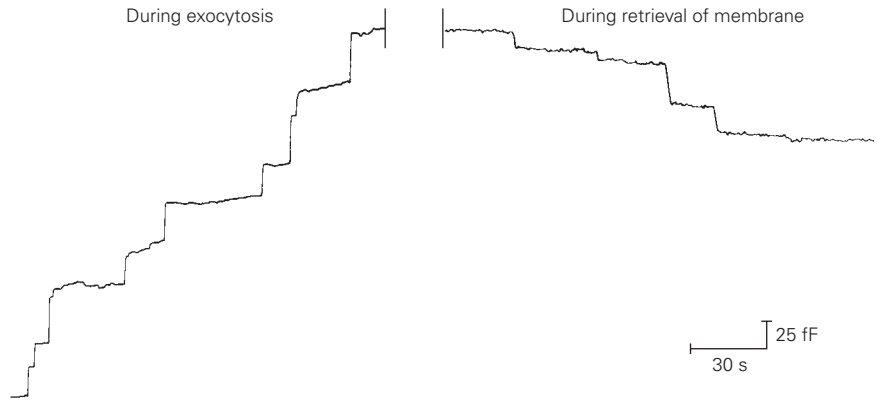
B. Synaptic vesicles release transmitter by fusing with the plasma membrane (exocytosis). Here, synaptic vesicles are caught in the act of fusing with the plasma membrane by rapid freezing of the tissue within 5 ms after a depolarizing stimulus. Each depression in the plasma membrane represents the fusion of one synaptic vesicle. In the micrograph at right, fused vesicles are seen as Ω-shaped structures.

C. After exocytosis, synaptic vesicle membrane is retrieved by endocytosis. Within approximately 10 seconds after fusion of the vesicles with the presynaptic membrane, coated pits form. After another 10 seconds, the coated pits begin to pinch off by endocytosis to form coated vesicles. These vesicles store the membrane proteins of the original synaptic vesicle and also molecules captured from the extracellular medium. The vesicles are recycled at the terminals or are transported to the cell body, where the membrane constituents are degraded or recycled (see Chapter 7).

A Mast cell before and after exocytosis of secretory vesicles



B Membrane capacitance during and after exocytosis of mast cell vesicles



C Retinal bipolar neuron terminal