

Neurotransmitter Release and Its Modulation

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The nature of synaptic interactions between cells is either excitatory or inhibitory. Activity in one cell either enhances or reduces activity in the target neuron. The strength of this influence and its duration are quite variable among synapses, and the mechanisms responsible for the postsynaptic effect are also variable. The classic synaptic action is to open ion channels, causing excitatory current to flow inward or inhibitory current to flow outward. Inhibitory synapses can also operate by increasing the postsynaptic conductance, shunting other synaptic responses. More recently, as documented in Chapter 9 and elsewhere in this volume, synaptic interactions have been observed in which ion channels are closed rather than opened, or in which voltage-dependent ion channels are modulated so as to change their voltage sensitivity. In all cases, the effect is to alter the postsynaptic neuron's electrical activity and its responsiveness to other inputs, thus changing the relationship between the cell and its environment.

Synaptic messages are transmitted by the presynaptic release of a transmitter substance that acts on postsynaptic receptors. The neurotransmitter is packaged presynaptically in vesicles, variable numbers of which fuse with the presynaptic membrane immediately following an action potential and release their contents into the synaptic cleft by exocytosis (Heuser et al., 1979; Zimmermann, 1979; Ceccarelli & Hurlbut, 1980). The evidence for this vesicle hypothesis of transmitter release is not completely compelling, and alternatives have been proposed (Tauc, 1982; Dunant & Israël, 1985). It is clear, however, that spike-evoked transmission involves the release of multiple packages of about 5,000 to 10,000 molecules of transmitter (Kuffler & Yoshikami, 1975), called quanta, within a millisecond or so after invasion of the presynaptic terminals by an action potential or other more slowly changing electrical signal.

In the absence of presynaptic activity, quanta are released spontaneously at a very low rate (typically about 1 per second at a neuromuscular synapse). These quanta evoke tiny postsynaptic responses, called miniature excitatory postsynaptic potentials (MEPSPs). Presynaptic depolarization increases the frequency of MEPSPs exponen-

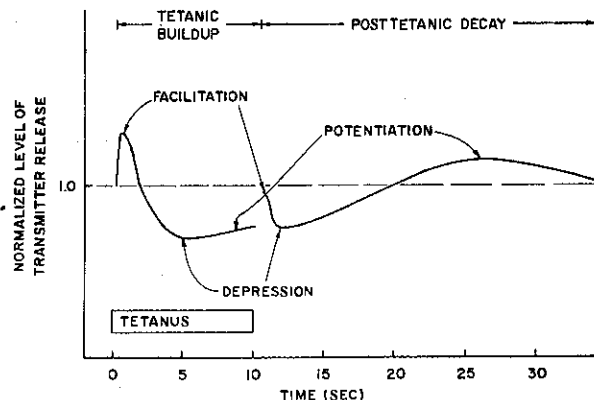
tially (Liley, 1956); thus, an action potential leads to an intense phasic acceleration of MEPP frequency. These quanta, released nearly simultaneously after an action potential, summate to form the full spike-evoked EPSP.

Synapses do not transmit information statically. Rather, their effectiveness depends in a sensitive and complex manner on the pattern of prior presynaptic activity. This plastic or modulatable property of synaptic efficacy has been appreciated since the earliest electrophysiological studies of synaptic transmission (Feng & Li, 1941) and has been termed *homosynaptic plasticity*. In addition, transmitter release can be regulated by the activity of another neuron, and the molecular mechanisms underlying such *heterosynaptic plasticity* are discussed in Chapter 10. This chapter discusses basic mechanisms of transmitter release and modulation by means of homosynaptic plasticity.

HOMOSYNAPTIC PLASTICITY

The effects of prior activity are quite varied at different synapses. Sometimes, a brief train of action potentials evokes rapidly increasing transmitter release to successive spikes. This effect dissipates equally rapidly, lasting between tens and hundreds of milliseconds (Magleby, 1973). The process is called synaptic facilitation. A minor component of facilitation, lasting several seconds, is sometimes distinguished and

Fig. 12.1. Sequence of events in homosynaptic plasticity at a "typical" synapse. Changes in the number of quanta released per impulse are shown for the successive spikes in a tetanus and for single test spikes at intervals following the end of the tetanus. During the tetanus, facilitation develops faster than depression, but depression is a larger effect and soon exceeds facilitation. A slowly developing potentiation appears as a reduction in tetanus depression. After the tetanus, the rapidly decaying facilitation can be seen at brief intervals after the last spike. Subsequently, depression decays, revealing posttetanic potentiation. The relative magnitudes and actual durations of facilitation, depression, and potentiation are quite variable among synapses and depend on the frequency and number of spikes in the tetanus (Hubbard, 1963).



called augmentation (Magleby & Zengel, 1976). At other synapses, successive spikes release less and less transmitter, until presynaptic spikes are almost ineffective on target neurons (Liley & North, 1953). This process, which is also reversible although somewhat more slowly than facilitation, is called synaptic depression. At some synapses, another process is evident in which spike-evoked release grows only very gradually during repeated presynaptic activity, and this slowly growing potentiation also lasts a long time, for minutes or even hours (Magleby & Zengel, 1975). Finally, at some synapses, long-term potentiation can last for hours or days (see Chapter 13), although in this case it is not clear whether the changes are in the presynaptic release of transmitter or in the response of the postsynaptic cell.

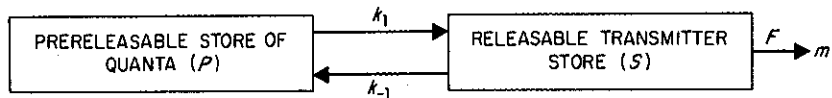
Many synapses display a mixture of these processes. Some neuromuscular junctions, for example, show superimposed facilitation, depression, and potentiation (Figure 12.1). The three processes accumulate during a train of spikes and afterward decay at different rates. Because potentiation requires a tetanus (train of many spikes) and can be observed in isolation only after the tetanus, when recovery from facilitation and depression have occurred, it is often referred to as posttetanic potentiation.

Depression

These signs of homosynaptic plasticity, or variation in strength of synaptic transmission dependent on prior activity in the same synapse, are interesting for two reasons: (1) they determine the information-transmitting characteristics of the synapse, and (2) they shed light on the physiological mechanisms underlying synaptic transmission. Synaptic depression, for example, is most pronounced when synaptic transmission is operating at high levels. Procedures that depress the amount of transmitter release by spikes also reduce the depression occurring to successive spikes (Thies, 1965). This suggests that depression is due to a depletion of transmitter stores available for release, with a slow recovery between impulses. A simple model of synaptic depression due to depletion (Figure 12.2) makes a number of testable predictions: (1) the magnitude of depression depends on the fraction of a transmitter store released by each impulse; (2) reducing this fraction, for example, by lowering external calcium concentration, reduces depression; (3) recovery from depression is exponential with a characteristic rate constant independent of the magnitude of depression; (4) tetanic depression accumulates exponentially at a predictable rate and to a predictable steady-state level, both of which are dependent on frequency of impulses, fraction of release, and the recovery rate. This model of depression provides an adequate description of the process at some synapses but not at others (Zucker & Bruner, 1977; Klein et al., 1980).

Facilitation

The mechanism of synaptic facilitation has also been the subject of intensive study. This is largely because it seems to be intimately tied to the process of transmitter release. To understand facilitation, we first need to review current ideas about the mechanism of release. When an action potential invades a nerve terminal, the depolarization of the terminal opens voltage-dependent calcium channels, which admit



k_1 = RATE OF REFILLING RELEASEABLE STORE (S)
 k_{-1} = LEAKAGE RATE OUT OF S
 F = FRACTION OF S RELEASED BY EACH IMPULSE
 $m(t)$ = NUMBER OF QUANTA RELEASED BY AN IMPULSE AT TIME t
 f = FREQUENCY OF STIMULATION

DURING A TRAIN:

$$\begin{aligned}
 dS/dt &= k_1 P - k_{-1} S - fFS, \quad m = FS \\
 m(t) &= FS_s - F(S_i - S_r)e^{-(fF + k_{-1})t} \\
 S_r &= \text{RESTING LEVEL OF } S = k_1 P / k_{-1} \\
 S_s &= \text{STEADY-STATE LEVEL OF } S = k_1 P / (fF + k_{-1})
 \end{aligned}$$

AFTER A TRAIN OF DURATION T :

$$\begin{aligned}
 m(t) &= FS_r + F(S_i - S_r)e^{-k_{-1}t} \\
 S_i &= \text{LEVEL OF } S \text{ AT END OF TRAIN} \\
 &= S_s - (S_r - S_s)e^{-(fF + k_{-1})T}
 \end{aligned}$$

Fig. 12.2. Mathematical outline of depression due to depletion of a releasable store of transmitter (Zucker & Bruner, 1977).

calcium into the terminal (Llinás et al., 1981a), where resting calcium concentration is very low—about 100 nM. Transmitter release is closely correlated to the calcium entry (Katz & Miledi, 1967a,b; Llinás et al., 1981b). Release by action potentials is very nonlinearly sensitive to the calcium concentration in the external medium, suggesting a cooperative action of several calcium ions in releasing transmitter once they have crossed the membrane (Dodge & Rahamimoff, 1967). Raising intracellular calcium by any means, such as microinjection, exposure to calcium-transporting ionophores, or fusion with calcium-loaded liposomes, elicits transmitter release whereas reducing calcium by opening calcium channels in a calcium-free medium reduces MEPP frequency. The idea that calcium is a necessary and sufficient agent in causing neurosecretion is called the calcium hypothesis of synaptic transmission (Figure 12.3).

The phasic release of transmitter after a spike is thought to be terminated by the rapid diffusion of calcium away from presynaptic sites of transmitter release. However, some of the calcium entering during action potentials will linger near release

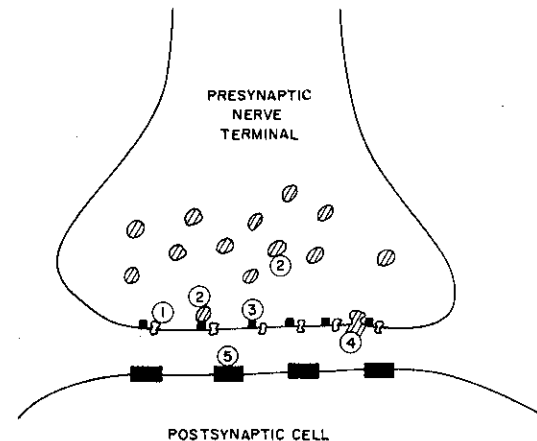
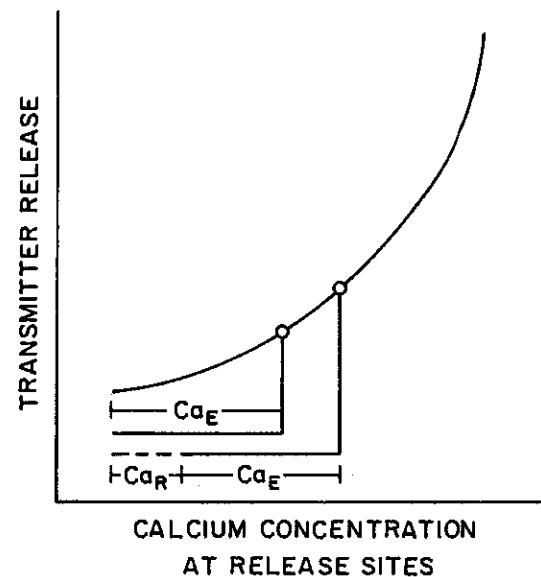


Fig. 12.3. Sequence of events involved in synaptic transmission. Presynaptic depolarization → calcium channels (1) open → calcium enters → active calcium rises → vesicles (2) fuse with release sites (3) → transmitter is liberated (4) → diffuses across synaptic cleft → binds to postsynaptic receptors (5) → postsynaptic ion channels open → postsynaptic current flows → postsynaptic potential generated.

Fig. 12.4. Nonlinear relationship between transmitter release and free calcium. Calcium entering during a spike (Ca_E) triggers more release in the presence of residual calcium (Ca_R) from prior activity.



sites, as the late phases of diffusional equilibrium are established or as the terminal begins to fill up with calcium (Zucker & Stockbridge, 1983). This "residual calcium" may be too little to elicit more than an acceleration of the frequency of spontaneously released MEPPs (Miledi & Thies, 1971; Zucker & Lara-Estrella, 1983). The influx of calcium accompanying a test action potential, however, will add to this residual calcium, and the peak calcium concentration at release sites will be higher than for isolated action potentials. Because of the nonlinearity of the relation between calcium and release, even a small augmentation in the peak calcium will release significantly more transmitter, leading to synaptic facilitation (Figure 12.4). This is the residual calcium hypothesis of synaptic facilitation.

Evidence for this hypothesis of synaptic facilitation comes from several experiments. Katz and Miledi (1968) showed that facilitation by an action potential requires calcium entry during the action potential. Furthermore, injecting calcium into the presynaptic terminal of the squid giant synapse facilitates release evoked by spikes without changing the spike waveform (Charlton et al., 1982). Charlton and Bittner (1978b) had earlier shown that facilitation is not due to a change in spike waveform or presynaptic afterpotentials. In addition, facilitation to a test pulse can be obtained even though the amount of calcium entering the cell during the pulse is not changed (Charlton et al., 1982).

Potentiation

The third form of homosynaptic plasticity, potentiation, has recently also come under experimental scrutiny. The magnitude of the effect depends on the presence of external sodium, suggesting that internal sodium accumulation causes potentiation (Levtov & Rahamimoff, 1980). This idea is supported by findings that microinjection of sodium ions, exposure to sodium ionophores, fusion with sodium-loaded liposomes, or treatments that encourage sodium accumulation, such as blockade of the sodium pump, all enhance potentiation (Charlton & Atwood, 1977; Meiri et al., 1981; Rahamimoff et al., 1978; Birks & Cohen, 1968; Atwood et al., 1975). The long duration of potentiation is believed to reflect the slow kinetics of sodium accumulation and removal. How internal sodium influences transmitter release is unknown, although it is often assumed that it operates by increasing intracellular calcium and is really a sodium-activated form of synaptic facilitation (Figure 12.5).

RECENT CHALLENGES TO THE CALCIUM HYPOTHESIS

The field of presynaptic physiology has always been exciting and full of ferment. The last 10 years have been no exception, as new techniques have been developed and applied to the study of transmitter release. Only in the last decade has a presynaptic terminal been voltage clamped, with consequent experimental control over ionic fluxes, especially calcium, intimately involved in synaptic transmission. Only in the last decade has the presynaptic calcium concentration been measured during synaptic transmission. And it is largely in the last decade that evidence has accumulated for the hypotheses of the processes involved in homosynaptic plasticity.

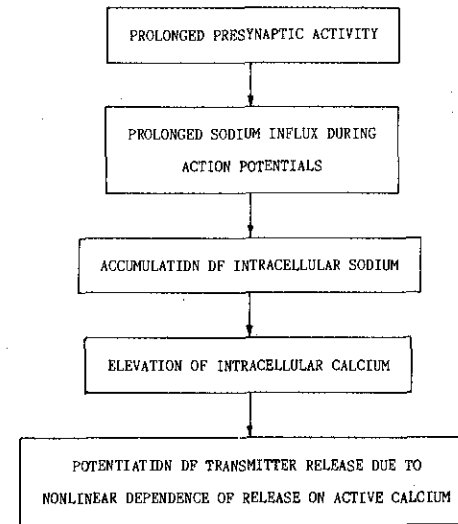


Fig. 12.5. Hypothetical scheme of events underlying synaptic potentiation.

As data have accumulated, however, their interpretation has been neither unambiguous nor unanimous. In fact, even the basic calcium hypothesis of transmitter release has come under attack. Recent experiments at the squid giant synapse (a huge junction in the stellate ganglion where the presynaptic terminal is up to 1 mm long and 50 μm wide) and at frog and crayfish neuromuscular junctions have suggested that, under certain circumstances, changes in presynaptic membrane potential can elicit transmitter release even in the absence of calcium influx or a change in calcium concentration at release sites. This can be called the voltage hypothesis of transmitter release. This issue is fundamental to our understanding of synaptic function, and an exploration of these recent challenges to the calcium hypothesis will occupy the remainder of this chapter.

The Squid Synapse

One indication of a direct role of membrane voltage in triggering neurosecretion came from observations at the squid giant synapse. Rodolfo Llinás and his colleagues (Llinás et al., 1981b) found that the same calcium influx can occur during pulses to low or high membrane potentials. Small depolarizations open few calcium channels with a large current per channel. Large depolarizations open all calcium channels, but as the calcium equilibrium potential is approached, the current per channel drops. According to the calcium hypothesis, a given calcium influx should elicit a given transmitter release, whatever the size of the pulse. On the contrary, Llinás and his associates found that a calcium current caused by a large depolarization elicits more release than the same calcium current caused by a small depolarization. This suggested a direct effect of membrane potential on the release process. However, as will

be shown later, this phenomenon is a simple consequence of diffusion of calcium ions in three dimensions following their entry through calcium channels (see below).

Neuromuscular Junctions

The other indications of a direct effect of potential on release come from experiments on neuromuscular junctions. An early observation, reported by Cooke, Okamoto, and Quastel (1973), was that depolarization can accelerate MEPSP frequency at frog neuromuscular junctions in the absence of added external calcium ions. This result was initially interpreted as an indication of a direct voltage effect, but later results indicated that the omission of a calcium chelator from the bath may have allowed external calcium to reach significant levels and that ions other than calcium might contribute to release in the absence of calcium (Hubbard et al., 1968; Kita et al., 1981).

More recently, Dudel (1983, 1984) and his colleagues (Dudel et al., 1983) have observed some surprising responses to depolarizing pulses at neuromuscular junctions that appear to contradict the calcium hypothesis of transmitter release. These findings can be readily explained, however, by the spatial inhomogeneity of stimuli applied through the large extracellular electrodes used in these experiments (Zucker & Landò, 1986). Furthermore, several other experiments described below provide direct evidence that transmitter release cannot be evoked by depolarization without calcium entry.

TRANSMITTER RELEASE REQUIRES CALCIUM INFLUX

Since the voltage hypothesis suggests that presynaptic depolarization can evoke release if intracellular calcium at release sites is already high, several methods have been used to elevate free calcium, and then depolarize terminals while blocking calcium influx. Hypertonic media accelerate MEPSP frequency, apparently by raising internal calcium in terminals uniformly throughout cytoplasm (Shimoni et al., 1977). This effect depends on the internal elevation of sodium ions (Muehnik & Venosa, 1969), suggesting a similarity to potentiation. The voltage hypothesis predicts that depolarization will elicit phasic release without calcium influx in hypertonic media. On the contrary, it was found (Zucker & Landò, 1986), in agreement with Shimoni et al. (1977), that depolarization reduces MEPSP frequency under such conditions, probably because of the efflux of calcium from terminals when calcium channels open in the presence of a reversed calcium gradient. Action potentials also fail to evoke release under such conditions.

In another series of experiments (Zucker & Landò, 1986), mitochondrial uncouplers were used to raise intracellular calcium. Such agents deplete terminals of ATP and cause mitochondria to release bound calcium. Uncouplers such as carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) cause a dramatic rise in MEPSP frequency (Glacoleva et al., 1970), but electrical depolarization of the terminals still fails to increase transmitter release. Cobalt ions are commonly used as a calcium channel antagonist. When calcium influx is blocked by substituting cobalt for calcium ions and raising internal calcium with CCCP, still no release is evoked by action potentials (Figure 12.6).

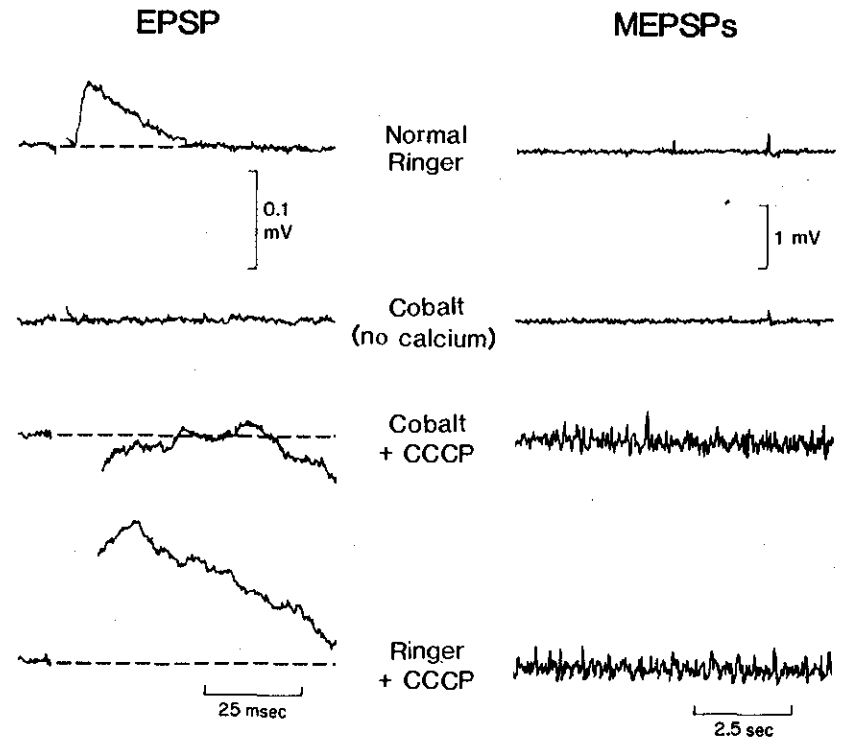


Fig. 12.6. Depolarization evokes no transmitter release without calcium influx, even when intracellular calcium at release sites is high. All traces are intracellular recordings from a muscle fiber in the leg opener muscle of crayfish. Replacing calcium in normal Ringer with cobalt blocks calcium influx and spike-evoked transmitter release. In CCCP, elevated free calcium increases MEPSP frequency from 1 per 8 sec to about 3 per second. CCCP also raises the threshold of the nerve, but when a larger stimulus is used to excite the nerve (causing a larger stimulus artifact), there is still no transmitter release and no EPSP. Restoring the normal calcium-containing Ringer restores transmission, which is facilitated by the elevated presynaptic calcium. Extracellular recordings from nerve terminals (not shown) indicated that the terminals were invaded by action potentials in all the solutions (unpublished results provided by L. Landò).

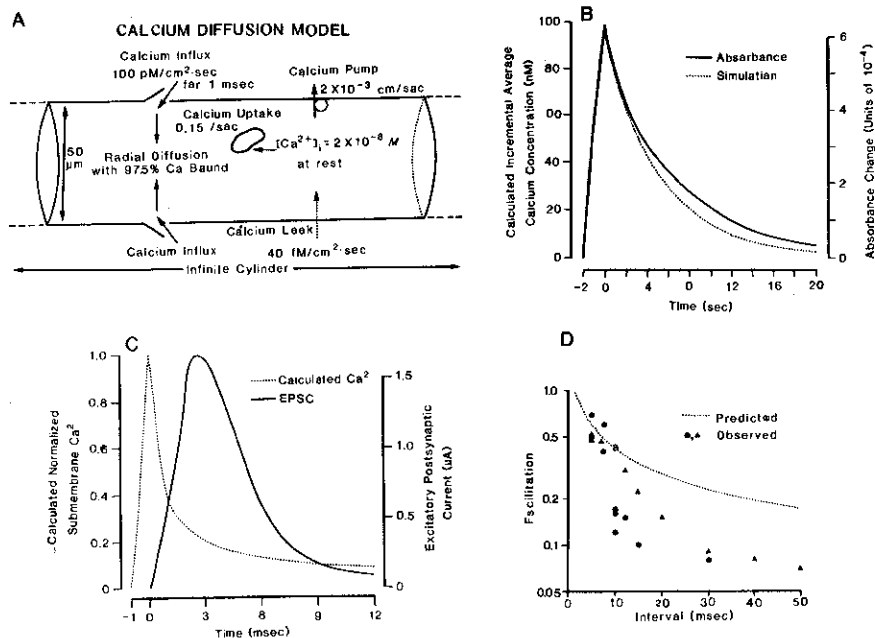
MODELS OF DYNAMIC BEHAVIOR OF ACTIVE CALCIUM AND TRANSMITTER RELEASE

The argument remains that the apparent voltage dependence of release in squid is inconsistent with calcium hypothesis of transmitter release. We shall now consider whether a mathematical formulation of the calcium hypothesis can account for this apparent discrepancy.

One-Dimensional Calcium Diffusion

The problem can be approached by considering the behavior of calcium entering a cylindrical terminal at the surface and diffusing radially inward following action potentials (Zucker & Stockbridge, 1983). This situation may be simulated by solving the diffusion equation in cylindrical coordinates with a brief pulsatile calcium influx at the surface corresponding to each spike. Intracellular calcium ions are known to be bound rapidly by cytoplasmic proteins, with two effects: (1) most of the calcium entering the terminal does not remain free, but is adsorbed to binding sites, and (2) diffusion is slowed substantially in relation to aqueous solutions. It is also known that calcium is extruded from cytoplasm by surface pumps and by uptake into intra-

Fig. 12.7. Simulations using a one-dimensional calcium diffusion model. Equation 4 from Zucker and Stockbridge (1983) was modified by dividing pump and influx terms by the ratio of bound to free calcium (correcting an error), and a term was included in Equations 2 to 4 to represent calcium uptake into endoplasmic reticulum. (A) Diagram of the system of equations; (B) total free cytoplasmic calcium during and after a 2-sec, 33-Hz tetanus. Calcium measurements by microspectrophotometric absorbance changes in a calcium-sensitive dye are compared to simulations. (C) The postsynaptic response (EPSC) to a presynaptic spike is compared to the time course of submembrane calcium raised to the second power. Events other than the rise and fall of submembrane calcium determine the time course of the postsynaptic response. (D) Simulations and experimental observations of facilitation (the fractional increase of the second response relative to the first) measured by the responses to two spikes separated by a variable interval.



cellular organelles. Finally, a square-law relationship between transmitter release and active calcium was assumed, based on measurements of the relationship between transmitter release and calcium current under voltage clamp (Charlton et al., 1982). Provisions for all of these processes were included in the simulation.

The initial results with this one-dimensional model were quite successful (Figure 12.7). The model predicts that the total free calcium in the terminal rises during a tetanus or single spike and declines afterwards with a half-time of several seconds. This corresponds to the kinetics of the change in presynaptic calcium measured using arsenazo microspectrophotometry (Charlton et al., 1982). The model predicts that phasic transmitter release would last only a few milliseconds or less, even if transmitter release is dependent only on the square of submembrane calcium. Higher stoichiometries lead to even more rapid release, but other processes subsequent to calcium action may be rate limiting. Finally, the model predicts that facilitation following one spike would peak at about 100% and decline with two time constants, about 6 and 60 msec, similar to experimental observation (Charlton & Bittner, 1978a). The success of these simulations was particularly significant because there were no free parameters. The calcium influx, geometrical dimensions, cytoplasmic binding efficiency, and extrusion and uptake rates were set according to independent measurements reported in the literature.

Simulation of Tetanic Responses

The effects of long tetani (100 spikes in 5 sec) were also simulated. The model succeeds in predicting an accumulating facilitation with several time constants, including one lasting seconds and corresponding to augmentation (Figure 12.8). This is caused by the filling of the terminal with calcium, so that a component of submembrane residual calcium is removed by extrusion processes. The faster phases of facilitation correspond to diffusion away from the membrane. Facilitation after a tetanus shows these same components, which correspond to similar time constants of decay following one spike. These characteristics are similar to those seen experimentally (Magelby & Zengel, 1982).

Simulations with this model, however, produce a major discrepancy with experimental data. After the last spike in the tetanus, the model predicts that submembrane calcium will remain higher than the peak active calcium reached in the first spike for an extended period. This would correspond to a posttetanic phasic transmitter release lasting well over 50 msec, which is never observed experimentally. Clearly, something is wrong with either the formulation of this model or the underlying assumptions (the calcium hypothesis of transmitter release).

Calcium Domains Near Calcium Channels

Simon and Llinás (1985) pointed out that calcium does not enter uniformly across the membrane, but rather through discrete calcium channels. Each calcium channel is surrounded by a little independent "calcium domain" (Figure 12.9A). Shortly after a spike, these calcium domains will collapse as calcium diffuses rapidly in three dimensions away from each channel. Within a few milliseconds, equilibration in the

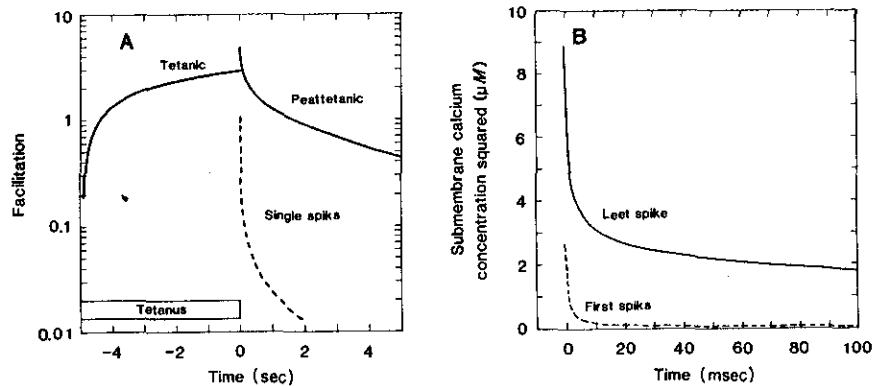


Fig. 12.8. Simulations of tetanic responses using a one-dimensional calcium diffusion model (adapted from Fogelson & Zucker, 1985). (A) The solid curve predicts facilitation of transmitter release to successive stimuli in a 5-sec, 20-Hz tetanus (rising phase) and to single test stimulus delivered at various times after the tetanus (falling phase). The dashed curve represents the decay of facilitation after a single spike. (B) Assuming transmitter release is proportional to the square of submembrane or active calcium, the time course of transmitter release is plotted for the first and last spikes in the tetanus. Release by the last spike is fallaciously predicted to continue for 50 msec at a level higher than the peak rate of release by the first spike. This problem is independent of the assumed cooperativity of calcium ions in releasing transmitter.

plane of the membrane will be established, and thereafter calcium will diffuse radially in one dimension, as in the model described previously. In this case, the peak calcium concentration near channel mouths at the end of a spike will be much greater than in the model with uniform calcium entry, although residual calcium will be the same. Thus, residual calcium will never approach the level of the peak calcium near release sites in a spike, and the major problem with the one-dimensional diffusion model disappears (Figure 12.9B). With a reduced ratio of residual to peak calcium, however, a higher calcium stoichiometry of release will be needed to account for facilitation. The mathematical formulation of these ideas requires the solution of the diffusion equation in three dimensions for an array of hundreds of thousands of calcium channels, with cytoplasmic binding and extrusion (Fogelson & Zucker, 1985).

Where Are Calcium Channels?

Before simulations with the three-dimensional model can be performed, some parameter choices must be made. The number of calcium channels can be determined by dividing the total calcium current (measured in squid synapses; Llinás et al., 1982) by the single channel calcium current (measured in snail neurons; Lux & Brown, 1984). The disposition of these channels is not very obvious. In early simulations

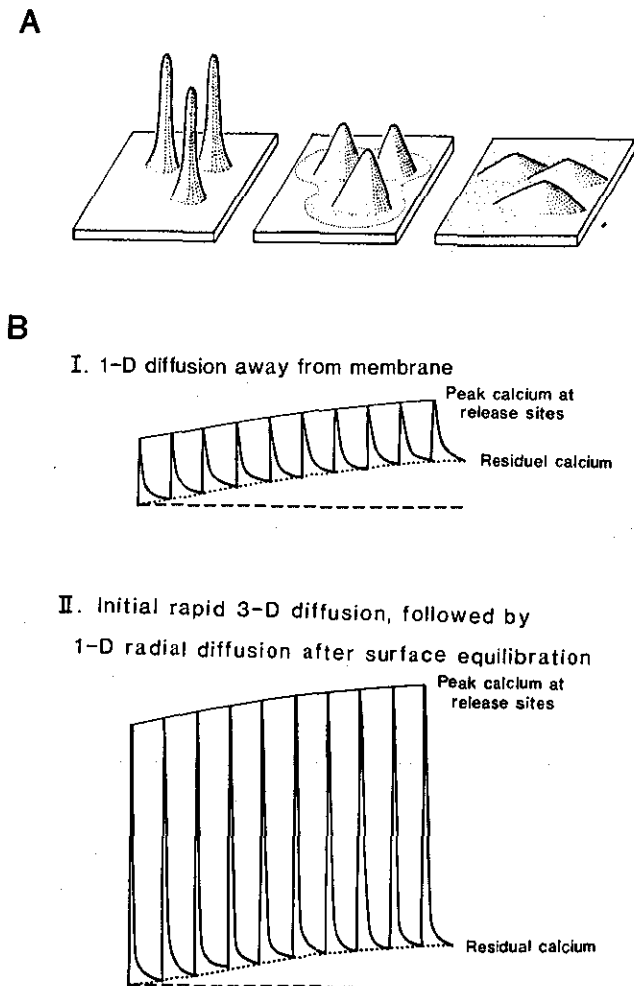


Fig. 12.9. Implications of calcium domains surrounding single calcium channels. (A) Sketch of hypothetical calcium concentrations near calcium channels at various times after the end of an action potential (adapted from Hartzell et al., 1975). Initially, calcium is confined to the regions surrounding channel mouths and diffuses away from them in three dimensions. Later, equilibration in the plane of the membrane is nearly complete, and subsequent diffusion is essentially one-dimensional (radial). (B) Comparison of models in which calcium influx is uniform (B-I) or is confined to discrete calcium channels (B-II). In the former case, residual calcium (dotted line) reaches a higher fraction of the peak active calcium than in the latter case.

calcium channels were distributed uniformly in the presynaptic membrane face in contact with the postsynaptic cells. Ultrastructural observations, however (Pumplin et al., 1981), indicate that synaptic vesicles and intramembranous particles thought to represent calcium channels are actually clustered into about 10,000 "active zones," each about $0.65 \mu\text{m}^2$ in area. Such an array of calcium channels has been used for more realistic simulations.

How far from calcium channels does exocytosis occur? The synaptic delay of 0.2 msec between calcium channel opening and exocytosis (Llinás et al., 1981b) allows calcium ions to diffuse only about 50 nm from channel mouths. Freeze fracture observations of exocytosis at frog neuromuscular junctions (Heuser et al., 1979) also show vesicle fusion occurring about 50 nm from putative calcium channels. If fusion occurred much closer than this, it would obliterate calcium channels in the process. Thus, transmitter release can be taken to be proportional to some power of calcium concentration 50 nm from calcium channel mouths. Since transmitter release depends on up to the fourth power of external calcium (Dodge & Rahamimoff, 1967; Katz & Miledi, 1970), and this dependence probably underestimates the stoichiometry or cooperativity of calcium action in releasing transmitter (Barton et al., 1983), a fifth-power relation between active calcium and transmitter release has been assumed for simulations.

Three-Dimensional Calcium Diffusion Simulations

Simulations with these parameter choices and other parameters (binding and extrusion) chosen as in the one-dimensional simulations provide gratifying fits to experimental results (Figure 12.10). The model simulates well the removal of total calcium from cytoplasm and facilitation during trains and after trains and single spikes. Most important, the chief problem with the one-dimensional model no longer exists. Transmitter release, as determined by the fifth power of calcium 50 nm from calcium channels, remains almost as short-lived after 100 spikes as after a single spike. Release decays in both situations with a half-time of less than 1 msec.

An unexpected result arises from simulations with a uniform distribution of channels. When calcium channels are not clustered in active zones, calcium does not diffuse rapidly away from each active zone in three dimensions; instead, equilibration in the plane of the membrane and the situation of the one-dimensional model are attained more rapidly. In these simulations, phasic transmitter release is prolonged to several milliseconds after the one-hundredth spike in a tetanus. The problem is similar to but not as exaggerated as the problem with the purely one-dimensional model. Only by boosting the rate of calcium extrusion by pumps to unphysiological levels can the model be made to account adequately for the removal of excess calcium from release sites. Apparently, the clustering of channels into active zones is necessary, to ensure the rapid diffusion of calcium away from regions containing release sites after long tetani. This may explain why the release machinery, consisting of calcium channels and synaptic vesicles, is clustered into separate active zones in all the chemical synapses studied so far.

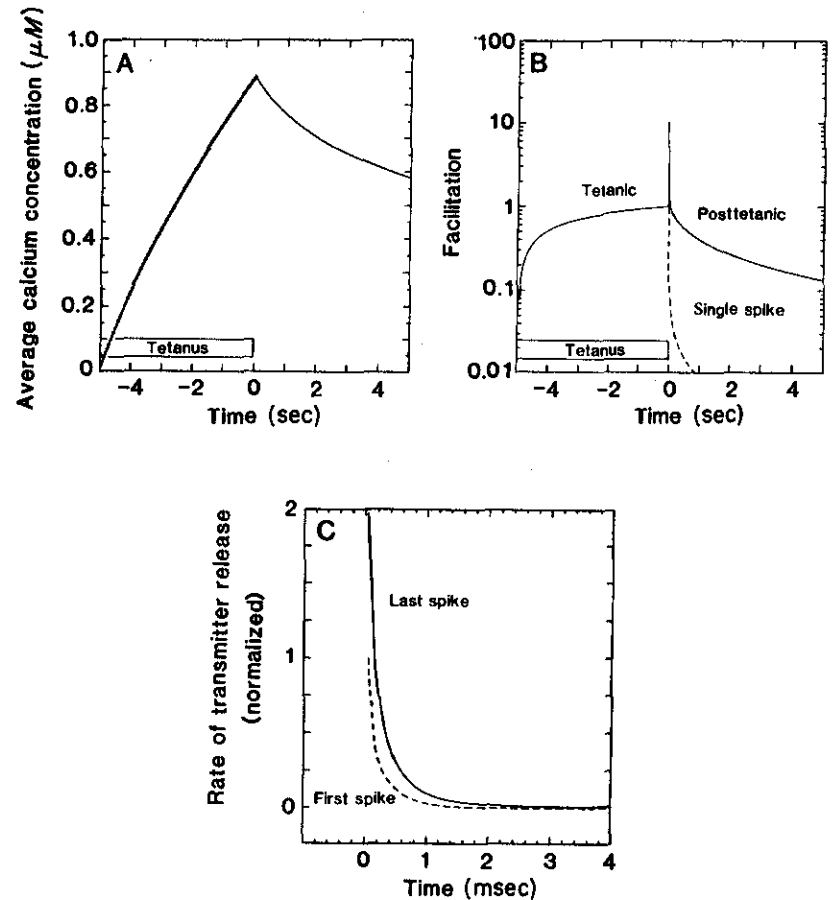


Fig. 12.10. Simulations of tetanic responses using the three-dimensional model (Fogelson & Zucker, 1985). (A) Prediction of average free cytoplasmic calcium concentration during and after a 5-sec, 20-Hz tetanus; (B) tetanic accumulation of synaptic facilitation and its posttetanic decay (solid curve) and facilitation following a single spike (dashed curve); (C) time course of decay of submembrane active calcium, 50 nm from calcium channel mouths in the center of active zones, for the first and last spike in the tetanus.

Relationship between Transmitter Release and Calcium Current

The three-dimensional model can also account for the finding that large depolarizations evoke more transmitter release than small ones, even when both depolarizations elicit the same calcium current (Llinás et al., 1981b; Smith et al., 1985). A third-order relation appears to exist between transmitter release and calcium current (Smith

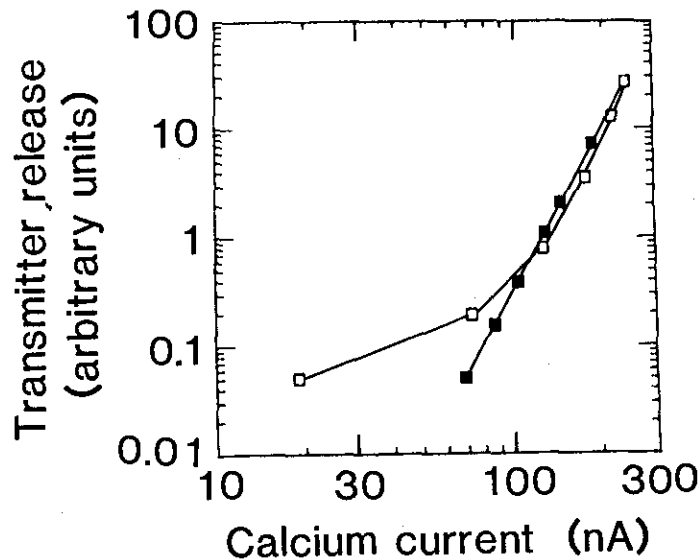


Fig. 12.11. Relationship between transmitter release and presynaptic calcium current at the end of a pulse, using different size depolarizations. The simulations are using the three-dimensional calcium diffusion model (Zucker & Fogelson, 1986). The open symbols indicate 2.5 msec pulses to 0 mV or less; the filled symbols indicate pulses to 10 mV or greater. Simulations of longer pulses are not possible, because they require calcium channels to reopen randomly in time and space during the pulse.

et al., 1985), somewhat lower than the calcium stoichiometry necessary to account for facilitation. It is also lower than the degree of calcium cooperativity suggested by the dependence of release on external calcium. Consideration of the effect of calcium domains, however, helps us to understand this result. If calcium domains during depolarizing pulses are entirely independent and nonoverlapping, then as more calcium channels are opened, more domains will be recruited, each releasing the same average number of transmitter quanta. A linear relationship between transmitter release and total calcium current would then result. (Actually, since larger depolarizations approach the calcium equilibrium potential, the single channel influx declines, and a less-than-linear relation results; Simon and Llinás, 1985.) This situation is analogous to the postsynaptic situation, in which the postsynaptic actions of individual quanta sum linearly, because of nonoverlapping quantal release sites, despite a nonlinear relation between transmitter concentration and postsynaptic current (see Hartzell et al., 1975).

If all calcium domains overlapped completely, then doubling the calcium current would double the number of channels opening and the submembrane calcium concentration. The relation between transmitter release and calcium current would then reflect faithfully the degree of calcium cooperativity in activating neurosecretion. In reality, when a few calcium channels are opened, their calcium domains remain en-

tirely distinct. As more channels open, their domains approach each other and begin to overlap. Thus, over much of the range of the relation between transmitter release and calcium current, a situation intermediate between complete independence and complete overlap should prevail.

It is impossible to intuit what the power of this relationship would be. The three-dimensional model, with calcium channels clustered in active zones, can predict this relationship for different size depolarizations (Figure 12.11). Assuming a fifth-order calcium cooperativity in releasing transmitter, the predicted power, 3.3, in the mid-range of depolarizations is strikingly close to that observed experimentally, from 2.4 to 3.5 (Smith et al., 1985).

Apparent Voltage Dependence of Transmitter Release

The model can also be used to predict the behavior of this relationship for very large depolarizations. The effects of brief pulses are simulated as opening calcium channels once during the pulse. Large pulses with a given calcium current at the end of the pulse differ from small pulses in two ways: (1) they open more calcium channels, with greater overlap of calcium domains, and (2) they admit less calcium through each channel during the pulse because of the lower driving force for calcium entry. These effects combine to make large depolarizations that elicit a given calcium current more effective than small depolarizations (Figure 12.11), as is seen in the experimental observations (Smith et al., 1985). Thus, the apparent voltage dependence of release is a natural consequence of the effect of variable overlap of calcium domains at different voltages and is not a sign of direct regulation of synaptic transmission by membrane potential.

CONCLUSIONS

These experiments and simulations leave us with restored confidence in the utility of the calcium hypothesis of transmitter release and its corollary, the residual calcium hypothesis of synaptic facilitation. Several challenges to the calcium hypothesis have withstood careful experimental and theoretical scrutiny. At present, no evidence exists that membrane potential can elicit transmitter release in the absence of calcium influx. The calcium hypothesis therefore remains the most useful model on which to base further experiments.

Although presynaptic voltage may not *elicit* transmitter release directly, recent experiments by Dudel (1984b) suggest that voltage may *modulate* release caused by calcium influx. These workers reported that small depolarizing (or hyperpolarizing) pulses, either immediately preceding or following a major depolarizing test pulse, enhance (or diminish) release by this major test pulse. However, they do not affect release evoked by a subsequent identical test pulse. Because the two major test pulses are identical, they should activate the same synaptic terminals under the electrode. The absence of an effect on synaptic facilitation suggests that the small pulses do not alter calcium influx evoked by the major test pulse or the resulting residual calcium.

Thus, presynaptic potential may modulate synaptic transmission without affecting calcium entry. More work on this interesting idea is needed.

It should be emphasized that, besides the possibility of a modulatory role of voltage, the calcium hypothesis does not account perfectly for all experimental observations. Certainly its formulation, even in the three-dimensional diffusion model, is not completely accurate. It is unlikely that exactly five or any other single number of calcium ions always cause a synaptic vesicle to fuse with the plasma membrane and release its contents. Neither calcium channels nor vesicle fusion sites occur in regular arrays, and calcium channels do not open synchronously, even in action potentials. In longer depolarizations, especially, the stochastic nature of different channels opening and closing repeatedly in random spatial and temporal patterns, as well as the local exhaustion of release sites and replenishment with releasable vesicles, is likely to affect significantly the properties of transmitter release. These imperfections may well be partly responsible for the imperfect fit of simulations to observations when fine details are considered. Nevertheless, the experimental results and simulations suggest that we are probably still on the right track, although nowhere near the final station.

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