FREQUENCY DEPENDENT CHANGES IN EXCITATORY SYNAPTIC EFFICACY

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INTRODUCTION

Chemical synapses are not static. The strength or efficacy of synaptic transmission is highly variable. One may measure synaptic efficacy by recording intracellularly the size of the postsynaptic potential (PSP, or EPSP at excitatory synapses) near the spike initiating zone. At some synapses, PSPs grow dramatically during repetitive stimulation to many times, even hundreds of times, the size of an isolated PSP. If this growth occurs quickly, within tens of milliseconds to a second during a tetanus, and decays afterwards just as quickly, it is called synaptic facilitation. If the growth in transmission develops gradually, requiring tens of seconds of continuous stimulation, it is referred to as potentiation or tetanic potentiation; its persistence and gradual decay after a tetanus is called post-tetanic potentiation (PTP). Enhanced synaptic transmission with a lifetime between potentiation and facilitation is sometimes called synaptic augmentation. At some synapses, potentiation can persist for hours or even days: then it is referred to as long term potentiation (LTP). In addition to the differences in timing that characterize facilitation, augmentation, potentiation and PTP, and LTP, there are differences in the physiological mechanisms underlying these processes.

Other chemical synapses are subject to fatigue or depression. Repeated activation leads to a decline in PSP amplitude during a tetanus, and severe depression can result in the virtually complete failure of transmission. Most synapses display a mixture of these dynamic behaviors. During a tetanus, transmission may rise briefly due to facilitation before it is overwhelmed by depression (60). If depression is not too severe, augmentation and potentiation will cause a partial recovery of transmission during the tetanus. After the tetanus, facilitation decays rapidly leaving depressed responses which recover slowly to the potentiated level, causing what appears as a delayed net potentiation or PTP (84). Finally, PTP or LTP decay during long periods of quiescence or low frequency activity.

These synaptic properties are often crucial to the information processing and response molding functions of neural circuits. Synaptic depression at mechanosensory synapses onto motor neurons causes the tonic habituation or lessening of graded responses like gill withdrawal in Aplysia (sea slugs) (35), and the phasic habituation or reduction in probability of nearly all-or-none responses like escape responses in fish,
crustacea, and insects (10,126,127). Depression at visual interneuron synapses onto higher interneurons results in local adaptation and alteration of receptive field in movement detecting visual neurons (97). Neuromuscular depression can cause the weakening of a response such as the crayfish tail flick (71).

Facilitation, augmentation, and potentiation shape the frequency sensitivity of synaptic transmission. Highly facilitating synapses respond effectively only to high frequency inputs. This has been shown to be important for the response characteristics of neurosecretory neurons and peripheral synapses (23,24,50,70). Long-term potentiation is particularly prominent in hippocampal pathways believed to be important in the consolidation of short-term into long-term memory. The existence of an associative enhancement of LTP (12,64), and an improvement in conditioning capability following induction of LTP (18) further implicate this synaptic process in changes underlying simple forms of learning.

The remainder of this chapter will focus on what is known of the physiological mechanisms underlying these forms of synaptic plasticity.

DEPRESSION

At many synapses, depression is the most prominent effect of repetitive stimulation when the synapse operates at a high level, initially releasing a lot of transmitter. Quantal analysis at neuromuscular junctions indicates that depression reflects a presynaptic reduction in the amount of transmitter released per impulse (44). Depression can be relieved by reducing the amount of transmitter released per spike, for example by reducing the external calcium concentration or elevating the magnesium concentration to block calcium influx at the nerve terminal (116). This property of depression suggests that it is due to a limited store of transmitter releasable by an action potential, which is not instantaneously replenished. Recovery from depression is roughly exponential, suggesting a first order process for renewing the releasable store. A simple model of depression has each action potential liberating a certain fraction of an immediately releasable store with subsequent recovery (74). This model provides a reasonably good approximation to the behavior of successive PSPs in a tetanus, but minor deviations from theoretical predictions are often observed. The fractional reduction in PSPs also declines as depression progresses (20), suggesting that the fraction of store released by a spike declined as the store was depleted. Perhaps the most easily released quanta (for example, vesicles well attached to release sites) are secreted first; those remaining are less readily released. Sometimes depression is less severe than expected during a tetanus (69), suggesting that the replenishment of the releasable store was not always first order, and may be boosted (mobilized) by excessive release of transmitter. One might expect depletion of transmitter to correlate with a reduction in vesicles, but depression develops faster and exceeds the reduction in vesicles, even those near the membrane (reviewed in 36). Despite these problems, the hypothesis that depression is due to the depletion of releasable transmitter stores remains the most popular for many neuromuscular junctions.

At some central synapses and neuromuscular junctions, depression has been found to be less dependent on the level of synaptic transmission than expected from a simple depletion model and to proceed during a tetanus in a manner inconsistent with predictions of such a model (33,132). In Aplysia central synapses, synaptic depression has been shown to be presynaptic in origin (34) and to correlate with a long-lasting inactivation of presynaptic calcium currents (66). This result contrasts with the squid giant synapse, where synaptic depression occurs in the absence of any reduction in presynaptic calcium
current (39). A recent analysis indicates that calcium channel inactivation in Aplysia is insufficient to account for the magnitude of synaptic depression. A new model (57) combines calcium channel inactivation and transmitter depletion to explain depression, and postulates a calcium-dependent mobilization of transmitter to explain the weak dependence of depression on level of transmission.

Although depression normally involves only a reduction in the number of quanta released by an impulse, at a fish central synapse depression is caused by a reduction in quantal size as well (19). It appears that at this synapse, releasable vesicles are not only depleted, but newly formed vesicles are also not entirely refilled between stimuli in a tetanus.

Finally, at some multiaction synapses in Aplysia, depression arises from postsynaptic desensitization of neurotransmitter receptors. A cholinergic interneuron in the abdominal ganglion activates excitatory and inhibitory receptors on a motor neuron to form a diphasic EPSP-IPSP. The excitatory receptor is particularly subject to desensitization, so that high frequency synaptic activation results in a brief excitation followed by persistent inhibition (117). The same effects are observed by iontophoresing acetylcholine onto the postsynaptic cell. Precisely the opposite situation prevails in synapses in the buccal ganglion in Aplysia. Here too, a cholinergic neuron activates two receptors on a postsynaptic cell and has a dual synaptic action. At this synapse, it is the inhibitory component which is most subject to desensitization, so that the synapse becomes purely excitatory at high frequencies of activation (56).

These examples serve to illustrate that depression is a multifaceted phenomenon, caused by a number of physiological processes at different synapses, and often having interesting consequences.

FACILITATION AND AUGMENTATION

Early Theories of Facilitation

Most chemical synapses display a short-term facilitation, such that successive spikes at high frequency evoke EPSPs of increasing magnitude. When depression is present it may mask facilitation, which will then only be evident when depression is relieved by reducing the amount of transmitter released by spikes. Whenever a quantal analysis has been done, facilitation has been shown to be presynaptic in origin, reflecting increasing amounts of transmitter release (reviewed in 128).

Early theories of facilitation invoked increases in spike invasion of presynaptic terminals or effects of spike afterpotentials in nerve terminals. These mechanisms have been shown not to be involved at central neurons (38), peripheral neurons (89), and neuromuscular junctions (29,60,129,131). It has also been suggested that spike broadening in nerve terminals, due to inactivation of potassium currents, might contribute to facilitation by increasing the calcium influx to successive action potentials (2,43). However, in the one preparation where this has been looked at critically, no evidence was found of spike broadening related to facilitation (133). Finally, it has been proposed that facilitation may be a property of calcium channels themselves (130), and successive depolarization of calcium channels in chromaffin cells does elicit facilitating calcium currents (58). However, calcium channels in Aplysia neurons (113) and presynaptic calcium channels at squid giant synapses do not have this facilitation property (39).

Residual Calcium Hypothesis

The most popular hypothesis of synaptic facilitation among current workers in the
field is the residual calcium hypothesis of Katz and Miledi (62,94,98). According to this hypothesis, facilitation is the natural consequence of a nonlinear relation between calcium and transmitter release and the fact that after an influx of calcium during an action potential some residual amount of the calcium that entered the nerve terminal will still be present at sites of transmitter release.

To understand this hypothesis, it is best to consider some numbers. The amount of transmitter released by an action potential depends on about the fourth power of external calcium concentration at a variety of synapses (47,48,61). This must be taken as a minimum estimate of the cooperativity of calcium action in releasing transmitter, because at low calcium concentrations the internal resting calcium interferes with this measurement, and at high calcium concentrations saturation of calcium flux through channels and of transmitter release interfere with the measurement (13,99). For these reasons, we will assume that transmitter release is determined by the fifth power of calcium acting at presynaptic intracellular release sites. This may reflect a mechanism for vesicle exocytosis in which the probability of vesicle fusion with the surface membrane increases rapidly as several calcium ions bind to sites either on the vesicle membrane or the vesicle attachment site on the plasma membrane (61).

Suppose the calcium concentration at release sites reaches one unit during an action potential and releases one unit of transmitter. Some time later (perhaps 10 msec), imagine that the calcium concentration has dropped to a residual level of 0.05 units. This residual calcium should cause release of transmitter at a rate of \((0.05)^5\) or one-three millionth the rate of transmitter release at the peak of the spike. At frog neuromuscular junctions in low calcium medium to prevent depression, a spike releases about 1 quantum within about 1 ms, so this residual calcium should cause spontaneous transmitter release to increase about \((3 \times 10^7) \times 1000/sec\) or about 1 quantum per hour! Meanwhile, a second action potential occurring at this time will generate a peak calcium concentration at release sites of 1.05, which will release 28% more transmitter than did the first spike. Calculations of this sort indicate that even after a tetanus, where residual calcium may reach significant levels such as 50% the peak of calcium concentration at release sites in an isolated spike, a large facilitation (660% in this case) will be accompanied by only a small acceleration in miniature EPSP frequency (31/sec). Such a correlation between facilitation and increased MEPS frequency has been observed in several (11,94,134), but not all experiments (124), and provides indirect evidence for the plausibility of a residual calcium hypothesis of facilitation. Another indication that calcium normally enters through calcium channels and results in a rise in intracellular calcium causing an increased MEPS frequency is the finding that in calcium-free media, tetanic stimulation results in a fall in MEPS frequency, presumably due to a drop in internal calcium as calcium exits through open channels (52).

**Experimental Support**

More direct evidence for this hypothesis comes from three sets of experiments.

1) Calcium is required for facilitation: Katz & Miledi (62) showed that not only transmitter release, but also facilitation, require calcium in the external medium. When calcium was raised after a conditioning impulse but before a test impulse, the first spike not only failed to release transmitter but also failed to facilitate release to the test impulse. It is not the release of transmitter by the first spike that permits facilitation to occur, however. Transmitter release is a statistical process, and at low levels of release spikes sometimes fail to release any quanta. Even following such
failures, a second spike is fully facilitated in calcium-containing medium (44,49). Apparently, calcium entry during the first spike causes facilitation whether or not the first spike releases transmitter.

2) Calcium elicits facilitation: Artificially raising presynaptic calcium, either by fusion with calcium containing liposomes (103), poisoning with inhibitors of calcium sequestration (4), or injection of calcium into giant presynaptic terminals (39), facilitates release by action potentials.

3) Residual calcium accumulates during repeated activity: Calcium concentration in presynaptic terminals has been measured spectrophotometrically with the indicator dye arsenazo III, and seen to rise about 1 uM during a tetanus of about 50 spikes (39,93).

Augmentation

This seems to be a longer lasting form of facilitation. It has been observed at neuromuscular junctions, synapses in sympathetic ganglia and cerebral cortex, and Aplysia central synapses (68,86,101,125). A slow phase in increased MEPSP frequency is seen which corresponds to this phase of increased evoked transmitter release (124). Augmentation also requires calcium entry, since tetani in calcium-free media do not elicit this increase in MEPSP frequency of intermediate duration (between facilitation and potentiation) (52).

Physical Models of Residual Calcium

The residual calcium hypothesis of facilitation has recently received additional support from attempts to explain the magnitude and time course of calcium at release sites necessary to account for facilitation and augmentation. The idea is that calcium crosses the presynaptic membrane into nerve terminals during action potentials (76,77) and acts at the surface to release transmitter. Calcium is bound to axoplasmic proteins (3,31) and diffuses rapidly toward the interior of the terminal, where it can no longer affect transmitter release. Calcium is slowly taken up into organelles (25), and extruded by surface membrane pumps (104). The magnitudes of calcium influx and calcium binding, and rates of uptake and extrusion have all been measured in squid giant axons and synapses and in synaptosomes (3,25,31,104). It ought to be possible, therefore, to solve the diffusion equation for a geometry appropriate to a nerve terminal (cylindrical) and with boundary conditions imposed by influx, binding, uptake, and extrusion, and predict the time course and magnitude of intracellular calcium gradients during and after nerve activity. Assuming a power-law dependence of transmitter release on submembrane calcium allows prediction of spike-evoked release and facilitation.

Initial simulations of these physical constraints used a one-dimensional model of radial calcium diffusion away from the surface, and assumed uniform calcium influx across the membrane (115,135). These simulations predicted the time course and magnitude of facilitation following one spike at squid giant synapses and frog neuromuscular junctions reasonably well, as well as the tetanic accumulation of total calcium and its decay as measured with arsenazo and the time course of spike-evoked transmitter release (55,135). However, the model predicted too high a post-tetanic residual calcium, compared to the peak submembrane calcium in one spike (55).

This defect was remedied in a subsequent more realistic model (55), in which calcium enters through an array of discrete channels and releases transmitter from release
sites at the surface near these channels. The brief synaptic delay requires that transmitter release occur near calcium channels before calcium equilibrates at the surface, when clouds of calcium ions still surround each open channel. After a spike, calcium diffuses away from each channel, and away from the clusters of channels, vesicles, and release sites called active zones (100). The peak calcium concentration at release sites in active zones in such a model is much higher than in the simpler one-dimensional diffusion model, and the residual calcium never reaches this level, even after a tetanus. Simulations of this model provide a quantitatively better, although still imperfect, fit to data on phasic transmitter release, accumulation of presynaptic calcium, and facilitation and augmentation at squid synapses and neuromuscular junctions. This model also has room for improvement, for example by considering the stochastic nature of the distribution of open calcium channels within an active zone.

**POTENTIATION**

Potentiation lasts even longer than augmentation, having a characteristic time constant on the order of one to several minutes at neuromuscular junctions and synapses in sympathetic ganglia, olfactory and hippocampal cortex, and Aplysia ganglia (8,85,101,105,109,118,125). At crustacean neuromuscular junctions, quantal analysis has shown potentiation to also be presynaptic in origin (122). Unlike facilitation and augmentation, the rate of decay of post-tetanic potentiation depends on the duration and frequency of the spikes in the tetanus, being slower for longer tetani (85,109).

Potentiation appears to arise from two sources. Potentiation is reduced but not abolished by stimulation in a calcium-free medium (52,106,119). This suggests that potentiation is due in part to calcium loading of presynaptic terminals during a tetanus and slow phases of calcium removal by pumps or uptake mechanisms that may become saturated at high calcium loads. The decay of PTP parallels those of post-tetanic calcium-activated potassium current and spectrophotometrically measured presynaptic calcium activity in Aplysia neurons (42,68), again suggesting that PTP is a consequence of a late component of residual calcium. The existence of a transition temperature in the temperature dependence of the decay of potentiation (110) and effects of alcohols on this decay rate (123) suggest that potentiation depends on some membrane process, such as extrusion of calcium by a membrane pump.

Part of potentiation is independent of calcium entry during a tetanus. This part is enhanced by procedures that augment sodium loading of nerve terminals such as blocking the sodium pump with ouabain, and reduced when sodium loading is minimized in low sodium media (8,21,22). Transmitter release can also be potentiated by exposing junctions to sodium-containing liposomes (103), introducing sodium with sodium ionophores (9,92) and injecting sodium into nerve terminals (37,121). It has been proposed that sodium which accumulates presynaptically during a tetanus causes potentiation by releasing calcium from intracellular stores (102) or reducing calcium extrusion by Na/Ca exchange (95). In that case, potentiation should be viewed as another consequence of increased residual calcium, dependent in some fashion on sodium accumulation. Although attractive as an hypothesis that unifies potentiation with facilitation and augmentation, there is no direct support for this notion.

**LONG-TERM POTENTIATION**

A very long lasting potentiation of synaptic transmission, with a lifetime of hours or even days, was first described in the hippocampus by Bliss and his colleagues
Brief bursts of presynaptic activity result in subsequently potentiated EPSPs in pyramidal cells. The process is pathway specific (5), but requires coactivation of several afferent axons (91). It therefore involves an interaction between presynaptic afferents, or a postsynaptic process occurring locally in small portions of the pyramidal dendritic tree and requiring more than one input. Additional evidence points to a postsynaptic trigger:

1) Postsynaptic depolarization coupled to weak afferent excitation generates LTP when neither alone is sufficient (65,120).
2) Postsynaptic hyperpolarization blocks LTP to an otherwise adequate stimulus (88).
3) Postsynaptic injection of the calcium chelator EGTA prevents LTP (80).
4) Antagonists of the N-methyl-D-aspartate class of glutamate receptor prevent LTP (40,41,51).

These results suggest that postsynaptic depolarization and activation of glutamate receptors are required to elicit LTP via a calcium-dependent pathway. One possibility is that calcium enters the postsynaptic neuron via glutamate receptor channels permeable to calcium (46,83) which only open when a pyramidal cell is depolarized (72,90,96) so as to remove a voltage-dependent magnesium ion block.

Several postsynaptic calcium receptors have been proposed as the target for calcium action. Calcium appears to activate a calcium-dependent proteinase called calpain (16) to degrade a protein called fodrin (111) which regulates cell surface receptors and cytoskeletal proteins (79). There is some evidence that fodrin acts to increase glutamate binding (14,15,112). However, an increase in glutamate binding in LTP has been questioned (82,108) and the calpain inhibitor leupeptin does not block LTP (107). Block of LTP by trifluoperazine suggests that calmodulin is involved in LTP expression (54). And the correlation of LTP with both changes in protein kinase C (1) and protein phosphorylation by kinase C (78), as well as the ability of kinase C or its activators to mimic LTP (59,75,87), have implicated this calcium-dependent kinase as a target of postsynaptic calcium.

The prolonged maintenance of LTP is even less well understood. Protein synthesis inhibition blocks LTP expression (114), suggesting the involvement of structural changes such as those observed in dendritic spines after LTP induction (45,73). Other evidence, however, points to increased presynaptic release of glutamate as the origin of potentiated transmission in LTP (26,53,81). A redistribution of synaptic vesicles during LTP (7) also indicates significant presynaptic changes. How these are triggered by a rise in postsynaptic calcium remains a total mystery.

Synaptic potentiation lasting for hours has also been observed at sympathetic ganglia (32) and crustacean neuromuscular junctions (17,121). Quantal analysis (17,67) and assay of transmitter release (30) show that LTP is due to an increase in transmitter release at both synapses. These presynaptic forms of LTP appear to be mechanistically unrelated to the LTP observed in the mammalian cortex. The crustacean neuromuscular LTP (called long-term facilitation) appears to be a prolonged component of PTP, due to presynaptic accumulation of sodium ions (121). LTP in sympathetic ganglia requires calcium influx (67) and may reflect a very slow component of calcium removal from highly loaded terminals.

CONCLUSION

This survey indicates quite a diversity of kinetic processes at chemical synapses. The efficacy of synaptic transmission is a highly plastic variable, subject to numerous
pre- and postsynaptic modulations sensitive to prior activity. These frequency-dependent changes often shape dramatically the pattern selectivity of synapses and the information transfer across them. Sensory processes such as adaptation and dynamic versus static sensitivity often reflect synaptic processes like depression and facilitation. Similarly, these same synaptic qualities may be expressed in behavioral habituation and in the recruitment of elements in a target neuron or effector pool. The various forms of potentiation have been implicated in longer forms of nervous system adaptations, such as learning and memory. With such behavioral consequences, these forms of frequency-dependent changes in synaptic efficacy are likely to continue to hold the interest of neurobiologists for some time to come.

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