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Structural Contributions to Short-Term Synaptic Plasticity

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Xu-Friedman, Matthew A., and Wade G. Regehr. Structural Contributions to Short-Term Synaptic Plasticity. *Physiol Rev* 84: 69–85, 2004; 10.1152/physrev.00016.2003.—Synaptic ultrastructure is critical to many basic hypotheses about synaptic transmission. Various aspects of synaptic ultrastructure have also been implicated in the mechanisms of short-term plasticity. These forms of plasticity can greatly affect synaptic strength during ongoing activity. We review the evidence for how synaptic ultrastructure may contribute to facilitation, depletion, saturation, and desensitization.

I. INTRODUCTION

The analysis of synaptic ultrastructure has provided critical insight into synaptic function. This approach began with electron micrographs of synaptic active zones and vesicles, which have formed the basis for current understanding of quantal properties of neurotransmitter release (25, 29, 88, 91). As knowledge of synaptic phenomena deepens, attention has turned to how ultrastructural features contribute to the variety of behaviors exhibited by central synapses (5).

Ultrastructural features contribute to synaptic diversity and influence the distinct forms of short-term synaptic plasticities present at different types of synapses. These forms of plasticity can lead to use-dependent enhancement or depression that persists for milliseconds to seconds (137). Such synaptic modification is thought to contribute to information processing by transforming presynaptic spike timing into fluctuations in synaptic strength (1, 17, 21, 23, 47, 69, 122). The variety of plasticities exhibited by different synapses reflects the variety of functions that synapses serve in extracting different features of presynaptic activity. However, the mechanistic bases for these forms of short-term synaptic plasticity are not well known. Here we review recent insights into synaptic transmission and short-term plasticity provided by ultrastructural characterization of synapses.

A. Forms of Synaptic Plasticity

Neurons typically influence the firing of their postsynaptic targets by releasing neurotransmitters that are contained in vesicles. However, because of short-term synaptic plasticity, each presynaptic action potential does not trigger the same amount of neurotransmitter release. Furthermore, the postsynaptic response to individual quanta can also change. Thus synaptic strength is highly dependent on the presynaptic pattern of activity. Many mechanisms contribute to such plasticity, including facilitation, post-tetanic potentiation (PTP), presynaptic depression, and postsynaptic desensitization. We first give a brief overview of these phenomena, which have been reviewed in detail elsewhere (73, 96, 135–137).

At many synapses, periods of elevated activity enhance the response measured in the postsynaptic cell. Multiple mechanisms including facilitation and PTP con-

tribute to such synaptic enhancement. Facilitation enhances the postsynaptic current (PSC) evoked by the second of two closely spaced presynaptic action potentials. An example of a facilitating synapse is the cerebellar parallel fiber to Purkinje cell synapse (Fig. 1*A*, *left*). When parallel fibers are stimulated twice in quick succession, the PSC following the second action potential is twice as large as the first (38, 67, 90). Facilitation typically persists for several hundred milliseconds following presynaptic activity. PTP is similar in that elevated levels of presynaptic activity enhance synaptic transmission, but PTP typically requires tens or hundreds of conditioning stimuli and lasts for tens of seconds (73). Both facilitation and PTP are produced by the build-up of calcium in the synaptic terminal during presynaptic activity (Fig. 1*A*, *right*) (4, 27, 28, 65, 137). Calcium enters through voltage-gated calcium channels during the first action potential (Fig. 1*A*, *right*, first pulse). The calcium channels close, and calcium equilibrates throughout the presynaptic terminal. These elevated calcium levels are termed "residual" calcium (Fig. 1*A*, *right*, residual Ca). When a second action potential arrives, residual calcium enhances the probability that a vesicle will be released (Fig. 1*A*, *right*, second pulse). PTP appears to last longer than facilitation because the higher levels of presynaptic activity result in a more prolonged presence of residual calcium (137).

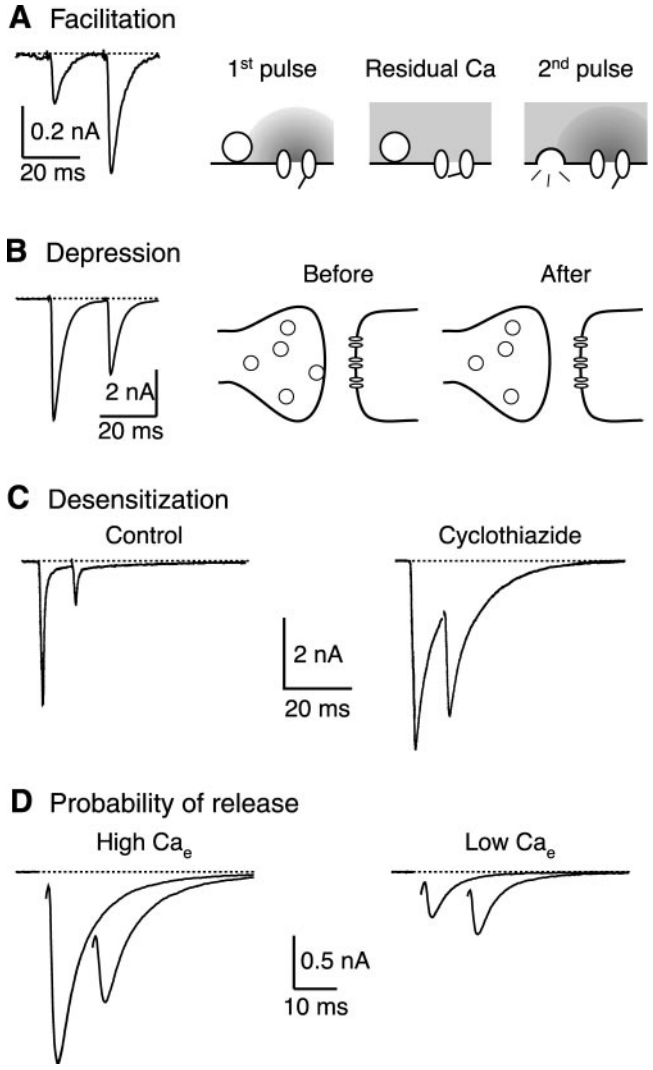

FIG. 1. Forms of short-term synaptic plasticity. *A*: facilitation. *Left*: postsynaptic currents (PSCs) at an example of a facilitating synapse, the cerebellar parallel fiber to Purkinje cell synapse. *Right*: proposed mechanism for facilitation. Calcium, represented by gray shading, flows in through voltage-gated calcium channels during the first action potential. After calcium channels close, the residual calcium remaining in the terminal is gradually pumped back out. When a second action potential arrives, the probability of release is enhanced by the extra calcium. *B*: depression. *Left*: PSCs at an example of a synapse that shows depression, the cerebellar climbing fiber to Purkinje cell synapse. *Right*: the proposed mechanism for the presynaptic form of depression, depletion. Before the first action potential, a releasable vesicle lies close to the presynaptic membrane. After the first action potential, the releasable vesicle has undergone fusion and is no longer available. When the second action potential arrives, there are no release-competent vesicles, so the synapse is depressed. *C*: desensitization. The auditory nerve synapse onto chick magnocellularis neurons shows strong depression (*left*). In the presence of 20 μM cyclothiazide, which prevents AMPA receptor desensitization, the amount of depression is reduced (*right*). In addition, cyclothiazide slows the time course of the AMPA receptor excitatory PSC, by both reducing desensitization and increasing receptor affinity for glutamate. *D*: switching between different forms of synaptic plasticity within the same synapse, by changing the external calcium concentration (Ca_e). The climbing fiber to Purkinje cell synapse can show different short-term plasticity under different conditions. In 2 mM Ca_e, the synapse shows depression (*left*). When the probability of release is reduced by changing Ca_e to 0.5 mM, the synapse shows facilitation (*right*). [*A*, *left*, and *B*, *left*, adapted from Xu-Friedman et al. (131); *C* adapted from Trussell et al. (121), with permission from Elsevier; *D* adapted from Foster et al. (48).]

Conversely, other synapses are depressed by elevated levels of presynaptic activity (37, 44). An example of a depressing synapse is the cerebellar climbing fiber to Purkinje cell synapse (Fig. 1*B*, *left*). When the climbing fiber is stimulated with a pair of closely spaced action potentials, the second PSC is decreased in amplitude (39, 67, 90). Depression can reflect multiple mechanisms, including vesicle depletion and postsynaptic receptor desensitization. Depletion arises when the first action potential releases a large fraction of the vesicles available for release (9, 42). Thus, when the second action potential arrives, there are fewer vesicles to be released, and the PSC is smaller (Fig. 1*B*, *right*, before vs. after). Recovery from depletion is thought to reflect that time required for the pool to be refilled, which can range from hundreds of milliseconds to tens of seconds (9, 42, 102, 103).

Postsynaptic responses are also reduced by desensitization, in which postsynaptic receptors exposed to neurotransmitter following the first action potential enter a nonconducting, desensitized state (66). Desensitization can occur at most ionotropic receptors, although its importance to short-term plasticity varies greatly at different synapses (63). The rate of recovery from desensitization

depends on the receptor subtype. For AMPA-type glutamate receptors, recovery from desensitization can be as fast as 10–50 ms (19, 58, 93, 120). An example of desensitization contributing to synaptic plasticity is found at the synapse made by avian auditory nerve fibers onto magnocellularis neurons (121). When the auditory nerve is stimulated twice, the second excitatory PSC (EPSC) is depressed (Fig. 1*C*, *left*). However, in the presence of cyclothiazide, a drug that prevents AMPA receptor desensitization (133), the time course of the EPSC is prolonged and the amount of depression is reduced (Fig. 1*C*, *right*).

How do we explain the observation that for the same experimental conditions some synapses facilitate while others depress? One clue comes from the fact that synapses can be manipulated to show either facilitation or depression by changing the external calcium concentration (Ca_e) (Fig. 1*D*) (72). Changing Ca_e has long been used to change the probability of release (P_r) by changing calcium influx (36). Synapses that depress when P_r is high (Fig. 1*D*, high Ca_e) can be transformed into facilitating synapses when P_r is low (Fig. 1*D*, low Ca_e). The dependence of short-tem plasticity on P_r suggests that when P_r is high the vesicle pool is significantly depleted following an action potential such that depression dominates, whereas when P_r is low, depletion is minimal and facilitation dominates. Facilitation and depletion probably both occur at all synapses, but which one dominates short-term plasticity at a given synapse depends on its P_r. Indeed, the type of short-term plasticity exhibited by a synapse is frequently used as an easily measured correlate of P_r. Thus facilitating synapses are believed to have low P_r, whereas depressing synapses have high P_r.

To explain the basis for short-term synaptic plasticity, the questions that we wish to address at a mechanistic level are: What determines P_r, and thus the balance of facilitation and depression? What determines the size of the vesicle pool, and how is it depleted? Why do some synapses show signs of desensitization whereas others do not?

B. Ultrastructural Features Implicated in Short-Term Plasticity

A number of ultrastructural features are thought to be involved either directly or indirectly with short-term synaptic plasticity. These are illustrated in Figure 2. In this example, a cerebellar climbing fiber (blue) contacts a Purkinje cell spine (pink) (Fig. 2*A*). The presynaptic axon contains a mitochondrion and a large number of vesicles. The area of contact between the axon and spine is a release site, as evidenced by active zone material, a widening of the synaptic cleft, and a postsynaptic density (PSD; arrowheads). The climbing fiber synapse onto the Purkinje cell consists of many release sites, each onto a separate spine. Within each active zone, a number of vesicles directly touch the presynaptic membrane (Fig. 2*B*). These vesicles, which are termed "docked," appear to be poised to fuse and release neurotransmitter to activate receptors on the postsynaptic cell (54, 114). They are believed to correspond to a readily releasable pool of

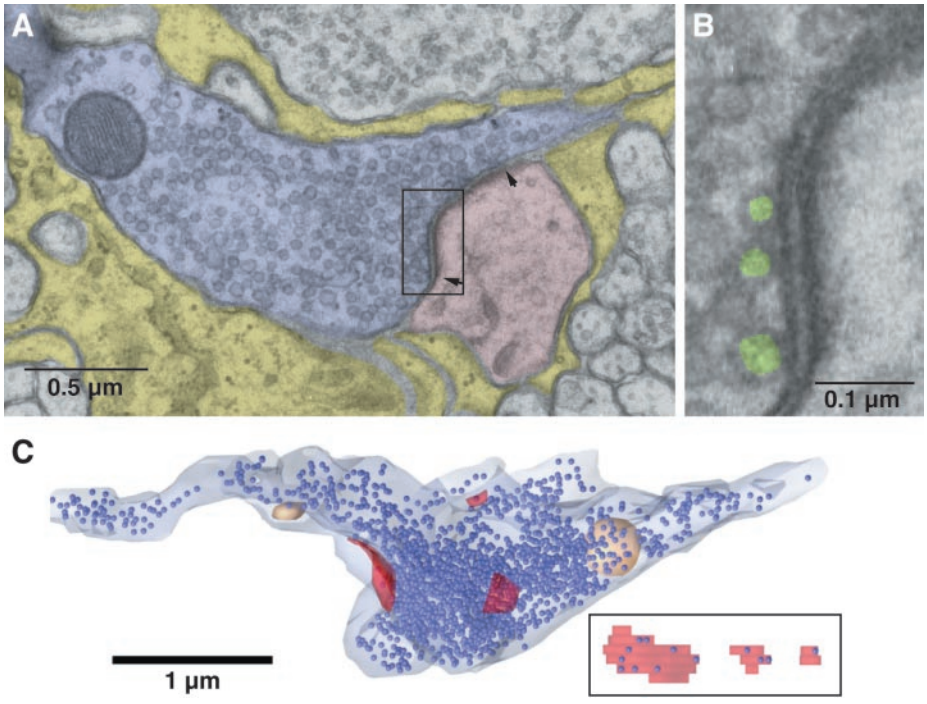

FIG. 2. Identifying ultrastructural features at synapses. *A*: single electron micrograph of a release site made by a cerebellar climbing fiber (blue) onto a Purkinje cell spine (pink). The release site is well surrounded by glia (yellow) and contains a mitochondrion. *B*: close-up of the boxed area in *A* showing docked vesicles (green). *C*: three-dimensional reconstruction using serial electron microscopy of a portion of a climbing fiber axon (light blue), with three release sites (red), mitochondria (tan), and all vesicles (dark blue). *Inset*: face-on views of release sites showing only docked vesicles (dark blue) across from the postsynaptic density (red). [Adapted from Xu-Friedman et al. (131).]

vesicles, which has been described using physiological techniques (11, 78, 98, 108, 112, 117, 126). The rest of the vesicles in the axon are probably too far from the membrane to participate in fast transmission and are believed to form a reserve pool for replacing vesicles that have been released. The entire climbing fiber synapse is ensheathed by glia (yellow), although the degree of ensheathment varies for different synapses. Because glia commonly express transmitter transporters (13, 99), their presence may indicate tight control over transmitter clearance from extracellular space.

Single electron micrographs can only provide a limited view of an entire synapse. Typically, a micrograph is 30–60 nm thick, but a release site may be much larger (for example, Fig. 2*B*). Furthermore, neighboring release sites may be up to 1 μm away (31, 104, 109, 131, 132). Therefore, to gain complete understanding of the parameters for each release site, and the spatial relationships between them, it is necessary to make serial electron microscopic reconstructions. The approach is to cut hundreds of thin sections, take pictures of each section, align the pictures, trace the structures of interest, and then use a three-dimensional rendering program to display the synapse in its entirety. The resulting image is displayed for a segment of a climbing fiber in which the axon is displayed in light blue, the vesicles in dark blue, the mitochondria in beige, and three PSDs in red (Fig. 2*C*). The high degree of variability in the sizes of the PSDs and the number of docked vesicles at these sites are apparent for the three release sites over this short segment of climbing fiber (Fig. 2*C*, *inset*).

Reconstructions make it possible to measure parameters that could affect different forms of synaptic plasticity. Presynaptic forms of short-term plasticity, such as facilitation and depletion, may be affected by presynaptic features such as the number of docked vesicles. Postsynaptic forms of plasticity, such as desensitization, may depend on ultrastructural features that will affect the glutamate signal and its reception, such as the shape of extracellular space, the degree of glial ensheathment, and the spatial relationships between release sites.

II. FUNDAMENTAL QUESTIONS WITH IMPLICATIONS FOR SHORT-TERM PLASTICITY

Before considering in detail how ultrastructure contributes to the mechanisms underlying short-term plasticity, it is necessary to address two fundamental questions that are crucial to the interpretation of physiological data. First, for a single release site, can an action potential evoke the release of at most one vesicle or can multiple vesicles be released? Second, are postsynaptic receptors saturated after release of a single vesicle? Knowing the answers to these two questions provides a framework for thinking about short-term plasticity. These issues are also important for the determination of the probability of release, which ultimately contributes to synaptic plasticity.

A. Mono- Versus Multivesicular Release

One hypothesis is that an action potential can trigger the release of at most one vesicle from a site; another is that an action potential can release multiple vesicles at a release site. The one-site, one-vesicle hypothesis arose from classic studies performing quantal analysis of release (68, 95). In these studies, the number of release sites estimated from statistical analysis of synaptic potentials matched the number of release sites determined using anatomical reconstruction. These experiments imply that a release site makes a fixed contribution to the synaptic potential, which was interpreted as being a single vesicle. Further support has come from studies of cultured hippocampal neurons (113).

However, much of this work was performed without knowledge of the state of postsynaptic receptor saturation. If receptors are saturated, then release of multiple vesicles could be difficult to distinguish from release of a single vesicle. Moreover, ultrastructural evidence indicates that many release sites contain multiple docked vesicles (54, 78, 81, 104–106, 109, 117, 131), so there is no a priori reason why they should not be able to release more than one vesicle. Thus the interpretation that only a single vesicle is released is worth examining in more detail.

One approach to looking for multivesicular release is to make a more thorough analysis of individual PSC amplitudes, to look for evidence of multiple release events at single sites. For example, at the synapses made by cerebellar inhibitory interneurons there are many overlapping, spontaneous PSCs (7). Statistical analysis suggested that there were more of these paired events than expected by chance, and so probably represented multiple release events from a single presynaptic neuron. Furthermore, interactions between these multiple events suggested they arise at the same release site. The second event in a pair had smaller amplitude than the first, particularly when the interval between them was very short. This suggested that the first PSC limited the number of receptors available for the second PSC, so both PSCs were sharing the same pool of receptors, indicating that these were desynchronized multivesicular release events. Even when a second event could not be resolved, larger PSCs elicited when P_r was high showed slower rise and fall times, suggesting that multiple events were underlying them (7). There is also evidence that multivesicular release occurs at synapses formed by cultured hippocampal neurons. Focal recordings of these synapses showed di-

rectly that bursts of spontaneous PSCs can arise at individual boutons (2). Furthermore, electron microscopy of quick-frozen hippocampal neurons showed multiple Ω figures per release site, which are believed to be vesicles caught in the act of fusing with the presynaptic membrane (2). This evidence is strongly indicative of multivesicular release.

Low-affinity receptor antagonists with fast kinetics (18) provide another approach to look for multivesicular release. Such antagonists block receptors well when transmitter levels are low (e.g., when a single vesicle is released). When transmitter levels are high (e.g., when multiple vesicles are released), the transmitter is able to compete with the antagonist during the PSC, and it is blocked less well. This means that if one PSC is affected more than another by a low-affinity antagonist, then it must have had a lower transmitter concentration. This behavior is not seen with high-affinity receptor antagonists, which block with the same effectiveness regardless of transmitter concentration. Thus low-affinity antagonists effectively reduce receptor saturation and help to uncover the contributions from additional vesicles (118).

This approach was used to study release at the cerebellar climbing fiber synapse (129). Climbing fibers were stimulated in normal Ca_e (2.5 mM) with pairs of pulses in the absence and presence of the low-affinity AMPA receptor antagonist γ-D-glutamylglycine (DGG). In control saline, the climbing fiber showed normal depression. In the presence of DGG, however, the first PSC was blocked to a lesser extent than the second. This suggested that the concentration of glutamate was lower for the second PSC. Because spillover appears not to occur at this synapse (129, 131), less glutamate release per site suggests that fewer vesicles were released per site on the second pulse, which necessarily means that multivesicular release can take place on the first pulse. It has been estimated that at least three or four vesicles are released on the first pulse (48, 129). When P_r was reduced by lowering Ca_e (0.5 mM), depression was reduced and both PSCs were blocked to similar extents by DGG. This suggested that under low P_r conditions, the amount of glutamate released by both pulses was the same, and perhaps the number of vesicles released per site had reached the minimum of one.

Optical techniques have also been used to examine multivesicular release. This has the theoretical advantage that one may directly examine release from single sites, rather than resort to more indirect arguments from whole cell, physiological recordings. This approach was used at CA1 pyramidal neurons, by loading them with calcium-sensitive indicators and imaging individual spines (20, 84). After stimulating presynaptic axons, *N*-methyl-D-aspartate (NMDA) receptors on the postsynaptic spine were activated, causing calcium influx and changes in fluorescence. Paired-pulse stimulation showed that responses to the first pulse were smaller than responses to the second pulse. Because these spines appear to receive input from only a single release site (53), the increase in amplitude at the second pulse was interpreted as arising from multivesicular release (84).

In summary, there is strong evidence that multivesicular release can happen at some synapses. This can be reconciled with data from the synapses that support the one-site, one-vesicle hypothesis. First, these synapses may be fundamentally different. Second, those synapses may show significant postsynaptic receptor saturation, which remains to be measured there. Nonetheless, it is clear that the hypothesis of one vesicle per site per action potential is not generally true.

B. Postsynaptic Receptor Saturation

There is considerable disagreement over the importance of postsynaptic receptor saturation. There is convincing evidence that some synapses show saturation whereas others do not, as detailed below. A further complication is that receptor saturation in principle is not all or none, even though it is usually framed that way. Partial saturation can occur such that two vesicles evoke a larger response than does a single vesicle, but the response is less than twice as large.

There are several lines of evidence for the lack of saturation at synapses. One of the first suggestions that there was no saturation came from quantal analysis. Although quantal size from classical studies at the neuromuscular junction was rather constant, quantal amplitude at many central nervous system neurons appears to be highly variable (8, 46, 50). This variability could arise from several sources. First, it could be due to the stochastic nature of channel opening, but this is unlikely to account for the full variability because the number of postsynaptic receptors appears to be high (43). Second, there could be variability between release sites, due to differences in the number of receptors (41, 83). Third, there could be variability in quantal content within individual release sites, either due to variability in vesicle size (57, 64, 134), in intravesicular transmitter concentration (50), or in the amount of transmitter that escapes the vesicle during fusion (16). If quantal content does contribute to quantal variability, it requires that the postsynaptic receptors must not be fully saturated. Otherwise, even a nonuniform population of vesicles would still be likely to occupy a large fraction of receptors and limit variability.

To demonstrate that the source of quantal variability is within a release site and not between release sites, the ideal approach would be to examine the quantal response at individual sites. This has been attempted in a number of studies by using minimal stimulation (8, 71, 76), by analyzing monosynaptic connections (110), and by loose-patching onto single boutons (46). The first two ap-

proaches can only be used to address this issue if it can be verified that only a single release site is being activated. In each of these studies, quantal variability was too high to be explained by the stochastic behavior of channels. This suggested that the receptors were not maximally occupied, and thus were not fully saturated. A further test of saturation is to puff exogenous glutamate onto release sites through a pipette, where a current larger than the largest quantal current would directly indicate the existence of additional receptors that could be activated. Care must be taken with this method to activate the identical pool of receptors and not extrasynaptic receptors. This has been done in hippocampal and spinal cord cultures, where the response to glutamate puffs is much larger and more consistent than the response to quantal release (71, 76, 125). This suggests that at these synapses, AMPA receptor saturation plays a minor role.

Another method for showing that quantal amplitude is far from the maximal response is to increase the quantal amplitude by overloading vesicles with transmitter. This can be done by patching onto presynaptic terminals with an electrode containing a high concentration of glutamate. A large synapse that can accommodate such an electrode is the calyceal synapse in the medial nucleus of the trapezoid body (MNTB). Ishikawa et al. (61) found that shortly after changing the contents of the patch electrode to high glutamate, there was an increase in the AMPA receptor-mediated quantal amplitude. This suggests that individual quanta do not completely saturate their postsynaptic AMPA receptors at the MNTB synapse.

Optical techniques are also useful in examining saturation at single sites. For example, hippocampal area CA1 pyramidal neurons were loaded with calcium-sensitive indicators and then their spines were imaged (74, 84). When the presynaptic axons were stimulated, glutamate activation of NMDA receptors led to calcium influx and fluorescence in the postsynaptic spine. By delivering pairs of pulses, it was clear that the fluorescence amplitude was greater after the second pulse than after the first. Because the spines appear to receive only single release sites (53), these changes in fluorescence amplitude corresponded to greater NMDA receptor activation, indicating that the receptors were not completely saturated by the first pulse (74, 84).

There are also several lines of evidence that saturation does play a role at some synapses. For example, at synapses made by cerebellar inhibitory interneurons, the second of two closely spaced spontaneous PSCs was statistically smaller than the first (7). If the second PSC had been independent of the first, it should have had the same amplitude on average. This suggests that the first release event was occupying a large fraction of postsynaptic receptors, leaving fewer for the second, which is convincing evidence for saturation, provided receptor desensitization was not a factor (116). In addition, at the climbing fiber, two closely spaced PSCs are differentially affected by the low-affinity AMPA receptor antagonist DGG (129). This happens because the amount of glutamate released per site on the first pulse is greater than that on the second. However, the true difference in the amount of glutamate is only detectable in the presence of DGG, indicating that when DGG is not present, AMPA receptors are saturated.

Although some of the variability in the apparent extent of saturation may reflect differences in experimental technique, it seems likely that there are real differences in the importance of saturation at different synapses. Therefore, it is useful to consider structural features at synapses that may contribute to this variability. Saturation can theoretically arise at a release site provided transmitter reaches concentrations in the synaptic cleft sufficient to activate a large percentage of receptors. Therefore, several factors probably contribute to saturation, including the concentration of transmitter in the vesicles (50, 61), the extent of vesicle fusion (16), the volume of the synaptic cleft, the rate of transmitter clearance, and the kinetics of receptors. Furthermore, saturation should be enhanced at synapses where receptors are located very close to the vesicle fusion site. Because ionotropic receptors appear to be concentrated at the PSD (82, 115), their distance from the fusion site will depend on the size of the PSD.

PSD size has been documented for several synapses using serial electron microscopic reconstructions. There is considerable variability between different synapse types (Fig. 3). Cerebellar climbing fiber and parallel fiber synapses are among the largest on average (0.14 μm^2 area, 0.21 μm radius) (131), and hippocampal CA3 to CA1 synapses are among the smallest (0.026 μm^2 area, 0.09 μm radius) (109). In addition, there is considerable variability within synapse types, as indicated by the standard deviation bars in Figure 3.

To understand how PSD size could affect saturation, it is useful to consider computational models of glutamate diffusion and AMPA receptor activation. Several modeling studies have suggested that the contents of a single vesicle of neurotransmitter can activate a significant fraction of postsynaptic receptors (43, 59, 123, 124, 130). To evaluate whether this level of activation can lead to saturation, we have taken the approach of using a highly simplified model with as many parameters as possible derived from the literature. More detailed stochastic models for transmitter diffusion and receptor activation have been explored but require far more parameters, many of which are not experimentally constrained. Therefore, we estimate the time course of glutamate in the synaptic cleft using a simple diffusion model (22). Transporters or other buffers, which are present at some synapses, could increase the rate of glutamate clearance, although probably not greatly since diffusion on this scale is already very fast.

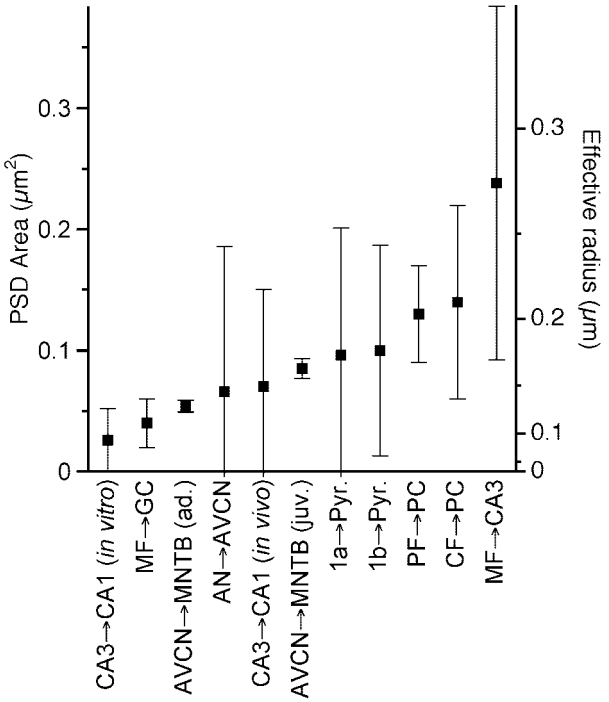

FIG. 3. Sizes of postsynaptic densities (PSDs) for several synapses measured using serial electron microscopic reconstruction. Markers indicate mean, and error bars indicate standard deviation. The left axis indicates the PSD surface area. The right axis indicates the corresponding radius for a circular PSD with the same area. Data on synapses made by CA3 pyramidal neurons onto CA1 pyramidal neurons (CA3→CA1) are from Shepherd and Harris (109) in vitro and from Harris and Stevens (53) in vivo. Data on synapses from cerebellar mossy fibers onto granule cells (MF→GC) are from Xu-Friedman and Regehr (132). Data on synapses made by juvenile and adult anteroventral cochlear nucleus neurons onto medial nucleus of the trapezoid body neurons (AVCN→MNTB) are from Taschenberger et al. (117). Data on auditory nerve end bulbs onto anteroventral cochlear nucleus neurons (AN→AVCN) are from Nicol and Walmsley (81). Data on synapses onto pyramidal neurons in pyriform cortical layers 1a (1a→Pyr.) and 1b (1b→Pyr) are from Schikorski and Stevens (106). Data on synapses made onto cerebellar Purkinje cells by climbing fibers (CF→PC) and parallel fibers (PF→PC) are from Xu-Friedman et al. (131). Data on synapses made by hippocampal mossy fibers onto CA3 pyramidal cells (MF→CA3) are from Chicurel and Harris (15).

Using this simple diffusion model, the glutamate concentration up to 0.5 μm from the fusion site reaches a peak within 10–200 μs and then decays (Fig. 4*A*). The peak glutamate concentration varies widely over this range of distances, from 100 mM under the fusion site to 100 μM at 0.5 μm away. We examine how these glutamate transients affect AMPA receptor activation across the PSD by passing them through a kinetic model of AMPA receptor activation (129) (Fig. 4*B*). We find that there are large differences in receptor activation over the first 0.5 μm from the fusion site. Receptors right under the fusion site are strongly activated, whereas receptors further away are activated significantly less.

To evaluate how these differences in activation might affect quantal size, we examined the receptor activity across different-sized PSDs. First, we consider how the site of vesicle fusion can affect quantal size. Because docked vesicles are distributed uniformly over the entire active zone (Fig. 2*C*, *inset*), release could potentially occur anywhere on the PSD. Previous modeling work has shown that the location of the fusion site can affect the size of the quantal PSC (43, 49, 123, 124, 130). We examined how PSD size could interact with fusion site to contribute to quantal variability. We assumed that receptors were distributed evenly across the PSD (115). We found that for a small PSD (0.1 μm diameter), activation across the PSD was fairly uniform whether a vesicle fused in the center or at the edge (Fig. 4*C*). By integrating activation across the PSD, we calculated the quantal PSCs for these two situations, and we found that the vesicle that fused in the middle of the active zone generated a quantal PSC that was 15% larger than a vesicle that fused at the edge (Fig. 4*D*, *left*). For a large PSD (0.5 μm diameter), the pattern of activation across the PSD was quite different (Fig. 3*C*). When the vesicle fused in the middle, only the receptors in the middle were strongly activated, whereas when the vesicle fused at the edge, only the receptors at the edge were strongly activated. Integrating across the entire PSD, the vesicle fusing at the center generated a quantal PSC that was 50% larger than the vesicle that fused at the edge (Fig. 4*D*, *right*). This suggests that PSD size could make a significant contribution to quantal variability.

We also compared the response to one vesicle released in the center of the active zone to the response to two vesicles. For the small PSD, the response to two vesicles was larger than the response to one vesicle (Fig. 4, *C* and *E*, *left*). However, when the response to two vesicles is compared against the linear prediction, i.e., two times the response to one vesicle, it was significantly smaller. Thus the small PSD showed partial saturation. However, when two vesicles are released in the center of the large PSD, more receptors are recruited further from the fusion site. The quantal PSC resulting from two vesicles was very close to the linear prediction, suggesting that saturation was nearly absent (Fig. 4*E*, *right*).

This modeling suggests that PSD size can significantly affect quantal variability and the amount of saturation. Because PSD size varies widely between synapses of different types (Fig. 3), this simple feature could account for some of the differences in saturation seen at different synapses. In addition, since PSD size varies within synapse type, saturation could vary for synapses of the same type. This is of particular concern, since larger synapses are easier to study experimentally using both physiological and optical methods, which could lead to sampling bias.

In summary, some synapses show evidence of postsynaptic receptor saturation, whereas others do not. Therefore, the relationship between the postsynaptic re-

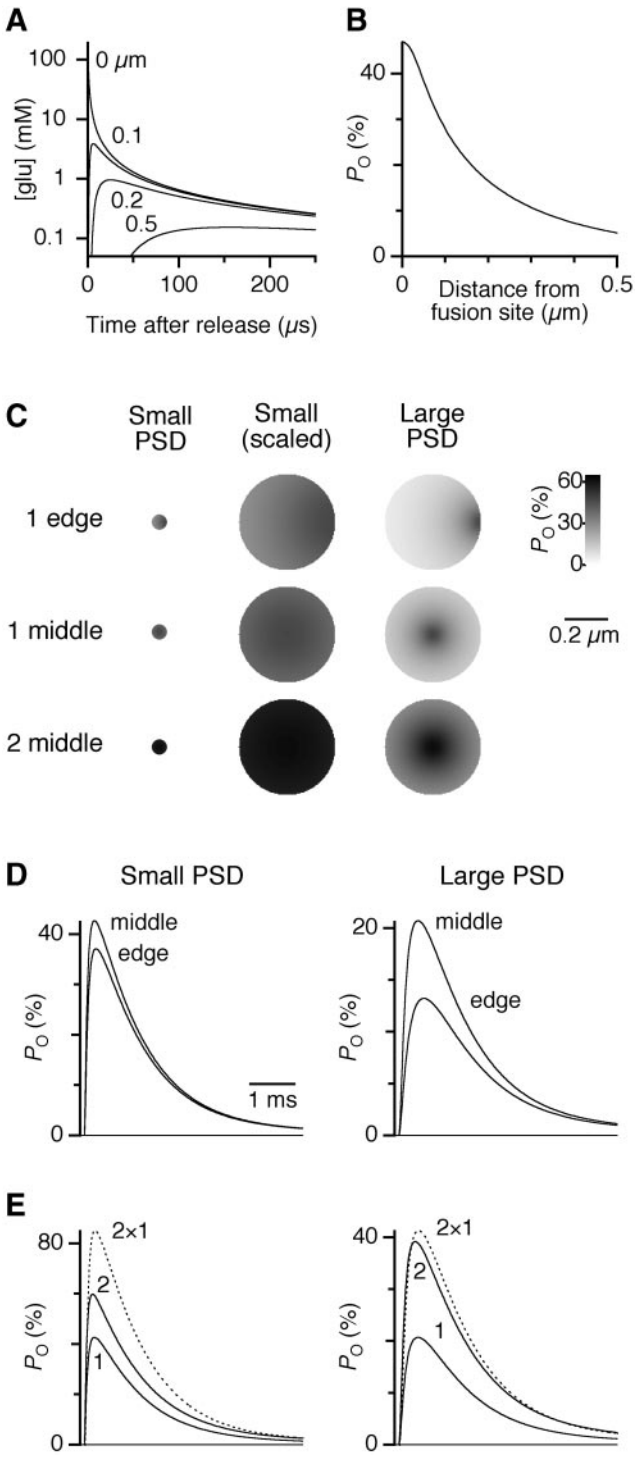

FIG. 4. Saturation of release is affected by the size of the PSD. *A*: model of glutamate diffusion. The concentration of glutamate over time is plotted at various distances from the fusion site. *B*: peak open probability (P_o) at various distances from the vesicle fusion site. Glutamate transients such as those shown in *A* were passed through a model of AMPA receptor activation (129). *C*: AMPA receptor activation across small and large PSDs. Peak P_o is shown in response to 1 or 2 vesicles released at the edge or the middle of the PSD. *D*: quantal sizes are more variable for larger PSDs. To measure quantal size, the open probability was integrated across the entire PSD for vesicles that fused in the center or at the edge. Model quanta are shown for circular PSDs of diameter 0.1 μm (area 0.008 μm^2, *left*) and 0.5 μm (area 0.20 μm^2, *right*). Small PSDs show responses of fairly constant amplitude regardless of fusion site. In contrast, large PSDs show greater sensitivity to fusion site. *E*: small PSDs show greater saturation. The response was modeled for 1 or 2 quanta being released in the center of the PSD. The response to 2 quanta is compared with twice the response to 1 quantum ("2×1"). Small PSDs (*left*) show partial saturation. The response to 2 vesicles is larger than the response to 1, but does not reach the linear prediction. Large PSDs (*right*) show little saturation. Glutamate diffusion is modeled as an instantaneous disk source on an infinite plane surface, given by: $C = 2C_0\rho/3Dt\lambda \exp(-r^2/4Dt)\int_0^\rho x \exp(-x^2/4Dt)I_0(rx/2Dt)dx$, where C is the concentration at time t and distance r from the fusion site, C_0 is the initial vesicle glutamate concentration (100 mM) (12, 97), ρ is the vesicle radius (25 nm), λ is the cleft width (20 nm), D is the diffusion constant (0.4 μm^2/ms) (85, 132), and I_0 is the modified Bessel function of the first kind of order zero (22). We calculate AMPA receptor activation using a kinetic model of Purkinje cell AMPA receptors at 34° (129). Nearly identical results were obtained using a second model based on chick magnocellularis somata (93).

sponse and the amount of presynaptic release may vary for different synapses. We propose that ultrastructural features such as PSD size could contribute to the amount of saturation at a synapse.

III. PROBABILITY OF RELEASE AND SHORT-TERM PLASTICITY

As we discussed in section IA, P_r is important for determining the amount of facilitation or depression at synapses. What is the basis for P_r? P_r is controlled by many factors, including the amount of influx through presynaptic calcium channels (36), the kinetics of calcium buffering (3, 40, 70, 100), the distance between the channels and the calcium sensor that triggers fusion (75), the identities of the proteins that make up the calcium sensor and release machinery (45), and the size of the releasable pool of vesicles (54, 105). P_r is closely related to two additional probabilities that govern release of these vesicles: the probability that an individual vesicle will fuse independently of the rest of the pool in response to an action potential (P_v), and the probability that an action potential will result in the fusion of any vesicle from the pool at a release site (P_s). The relationship between these two probabilities depends on whether multivesicular release is present and whether one vesicle can saturate postsynaptic receptors, as explained in the following cases.

In *case 1* (release is monovesicular), the probability of release is represented by P_s. Although saturation may limit the amplitude of the quantal PSC, it will not affect the determination of P_s. Because only a single vesicle is released, depletion will be minimal, unless the readily releasable pool is very small, as explained in section IV.

In *case 2* (release is multivesicular, with complete saturation), release of one vesicle cannot be distinguished from release of more, even though these reflect different values for P_v. For this case the relationship between P_v

and P_s is given by $P_s = 1 - (1 - P_v)^n$, where n is the number of vesicles in the releasable pool. Synapses with this characteristic would be expected to show prominent depletion when P_v is high, although the extent of depression would be shaped in part by saturation (see sect. v*A*).

In *case 3* (release is multivesicular, without saturation), the response to each vesicle is independent of the others, so each vesicle is effectively its own release site. Therefore, the relevant probability of release is actually P_v. When P_v is high, there is depression, and when P_v is low, there is facilitation.

Because the issues of multivesicular release and saturation are unresolved at most synapses, it is unknown whether the relevant probability is P_s or P_v. An additional complication is that if a single vesicle leads only to partial saturation, neither *case 2* nor *case 3* is applicable and the relationship between P_s and P_v is more complex. Therefore, in some cases we will use the more generic term of probability of release, P_r.

The implications of *cases 1* and *2* led to the advancement of a hypothesis that the anatomical correlate of the readily releasable pool is the set of anatomically docked vesicles, which thus determines P_r (35, 54, 105). A release site with many vesicles would have a greater likelihood of releasing at least one vesicle than a release site with few vesicles (Fig. 5*A*). This hypothesis requires that either release is monovesicular or saturation is significant. Under these conditions, the critical parameter is the probability that at least one vesicle at a site is released (P_s), rather than the probability of any individual vesicle (P_v).

This hypothesis has the advantage that the number of docked vesicles can be counted in central synapses by doing serial electron microscopy and identifying which vesicles are directly touching the presynaptic plasma membrane (Fig. 2*B*). It is generally believed that these vesicles are the only ones that could be released in response to an action potential because the other vesicles are simply too far away to be mobilized within the short time scale of vesicle fusion (114). More distant vesicles probably consist of two different pools, those that could replace vesicles that have been released and those that are still in the process of recycling by loading with transmitter or assembling the release machinery.

It is more difficult, however, to measure the readily releasable pool of vesicles physiologically. In the ideal situation, one could specifically trigger release of the entirety of the readily releasable pool and measure its amplitude. However, physiological approaches to identify the release-ready and reserve pools are complicated by the difficulty of fully depleting a pool without any replenishment of that pool. One solution is to trigger release of a large number of vesicles and break the time course of release down into separate components. The component with the fastest time course is then taken to represent the readily releasable pool, and later components may represent different pools of reserve or replenished vesicles. This general approach has been used, where the specific techniques to release vesicles include hypertonic sucrose application, trains of extracellular stimulation, calcium uncaging, and direct depolarization of the presynaptic terminal (11, 78, 98, 108, 112, 117, 126). Release can then be monitored using PSCs or presynaptic capacitance. Because these approaches typically involve release of huge

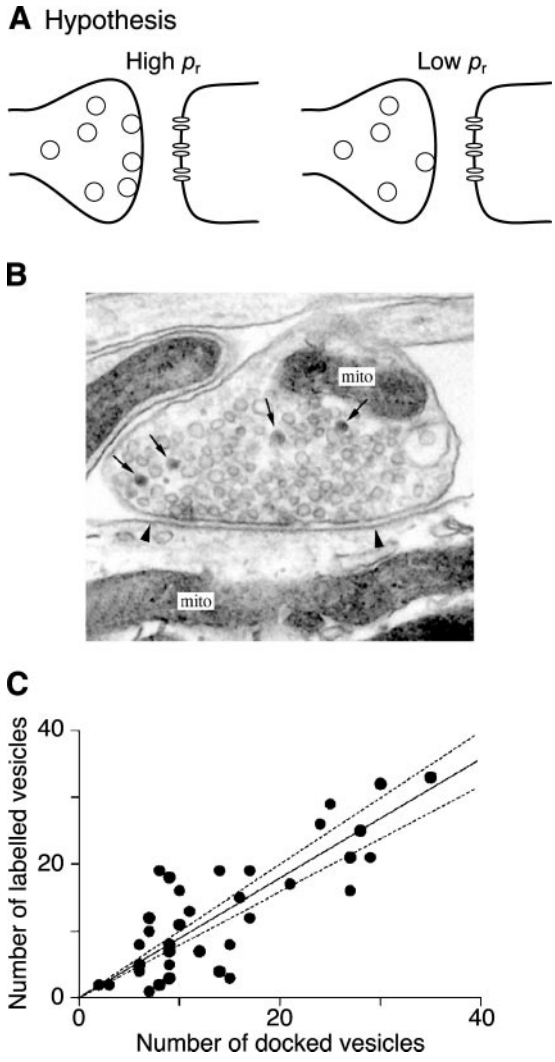

FIG. 5. Evidence that the number of docked vesicles determines the probability of release (P_r). *A*: model for how the number of docked vesicles determines P_r. A high P_r synapse has many docked vesicles, increasing the likelihood that at least one vesicle fuses. A low P_r synapse has few docked vesicles. *B*: quantifying the readily releasable pool, P_r, and the number of docked vesicles. Labeled vesicles (arrows) represent vesicles that were taken up following 2 s of 20-Hz stimulation in the presence of FM1–43. The number of labeled vesicles can be used to calculate P_r by dividing by the number of stimuli. The number of docked vesicles is also counted. Arrowheads mark the extent of the PSD. *C*: correlation between the number of anatomically docked vesicles and the number of vesicles labeled by FM1–43 for a number of synapses. Line is the correlation (slope = 0.89). [*B* and *C* adapted from Schikorski and Stevens (107), with permission from Nature Publishing Group (www.nature.com).]

numbers of vesicles, PSC data could be complicated by the effects of receptor saturation and desensitization.

The correlation between the docked vesicle pool and the readily releasable pool has been tested in retinal bipolar neurons. These cells are nonspiking and have graded release of transmitter, so the concept of P_r does not apply. However, these cells make a very useful test of part of this hypothesis because of their simple, regular synaptic ultrastructure. The bipolar cell has a ribbon-type synapse, where vesicles are tethered along an electron-dense structure (94). The size of the releasable pool was measured using presynaptic capacitance measurements, which resolved the presence of two pools of vesicles based on the rapidity with which they could be released. The immediately releasable pool contained 1,100 vesicles (77), and the reserve pool contained 4,600 (126, 127). These measurements have been compared against two separate, anatomically defined pools. The first anatomical pool of vesicles is docked at the base of the ribbon next to the plasma membrane and contains 1,000–1,400 vesicles (127), which correlates well with the immediately releasable pool. The second anatomical pool constitutes the vesicles tethered further up the ribbon away from the plasma membrane and contains 3,950–5,750, which correlates well with the reserve pool. Thus the results from the retinal bipolar cell support the identification of anatomical pools of vesicles with physiologically defined responses.

Applying this same approach to nonretinal central synapses is complicated by their anatomy and by difficulty in measuring the pool size with physiological methods. Few central synapses show regular active zone structures like the presynaptic ribbon, where a hierarchy of vesicles is strongly suggested. For practical considerations, then, only two classes of vesicles are currently distinguishable. The first consists of anatomically docked vesicles that directly touch the presynaptic membrane. These vesicles are probably readily releasable. However, it is possible that additional priming steps are necessary before they can be released, which are not visible in simple electron micrographs. The second class is all the other vesicles present in the presynaptic terminal, which are hypothesized to make up the reserve pool.

The greatest support for the link between readily releasable pool size and P_r comes from studies of synapses made between cultured hippocampal neurons. This population of synapses shows considerable variability in short-term plasticity. To determine the source of this variability, Stevens and colleagues (35, 80, 98, 105) have explored methods for determining P_r, the size of the readily releasable pool, and the number of docked vesicles. The most refined approach to date was taken by Schikorski and Stevens (107). They labeled the readily releasable pool of vesicles by stimulating synapses with a long train in the presence of the dye FM1–43, which could be photoconverted to allow counting of the vesicles using electron microscopy (Fig. 5*B*). The number of FM1–43-labeled vesicles correlated tightly with the number of anatomically docked vesicles at the same release sites and varied from 2 to 35 (Fig. 5*C*). Thus release sites with low P_r had fewer FM1–43-labeled vesicles, and they also had fewer docked vesicles.

While this hypothesis appears to account for variability in P_r within a population of synapses of the same type, its ability to explain differences in P_r between different types of synapses has had mixed success. One example that supports the hypothesis is found in the pyriform cortex. There, synapses onto pyramidal neurons in layer 1a facilitate, and synapses in layer 1b do not (10). This suggests that P_r is higher in layer 1b than in 1a, so layer 1b release sites should have more docked vesicles. Measurements made using serial electron microscopy found that layer 1a release sites had 16 ± 17 vesicles, whereas layer 1b release sites had 27 ± 24 vesicles, supporting the hypothesis (106).

However, other synapses have not fulfilled the predictions of this hypothesis. One counterexample comes from the cerebellum. Cerebellar Purkinje cells receive synapses from parallel fibers, which facilitate, and climbing fibers, which depress. Under identical experimental conditions, the value of P_r has been estimated for these synapses to be <0.1 for parallel fibers and 0.4–0.9 for climbing fibers (33, 111). According to the hypothesis, one might therefore expect to find four to nine times as many docked vesicles at climbing fiber release sites compared with parallel fiber release sites. However, serial electron microscopic reconstructions of climbing fibers (Fig. 2*C*) and parallel fibers (Fig. 6*A*) showed that they had the same average number of docked vesicles, 7 ± 3 for the climbing fiber versus 8 ± 3 for the parallel fiber (Fig. 6*B*) (132).

Another counterexample has been documented at crustacean motor neurons (78). Two different motor neurons terminate on the same muscle, but they have very different properties. One is highly facilitating (tonic), equivalent to low P_r, and the other is highly depressing (phasic), equivalent to high P_r. According to the hypothesis, the tonic motor neuron should have fewer docked vesicles and a smaller readily releasable pool than the phasic motor neuron. However, the opposite was true. The tonic neuron had a physiologically determined releasable pool that was twice the size of the phasic neuron (130 ± 23 vs. 58 ± 6). In addition, the tonic motor neuron had many more anatomically docked vesicles than the phasic motor neuron (11 ± 0.5 vs. 4.4 ± 0.3) (78).

These results suggest that the number of docked vesicles at a release site can contribute to the nonuniformity in the probability of release at different sites for a particular type of synapse. However, when comparing different types of synapses, the number of docked vesicles does not account for differences in either the prob-

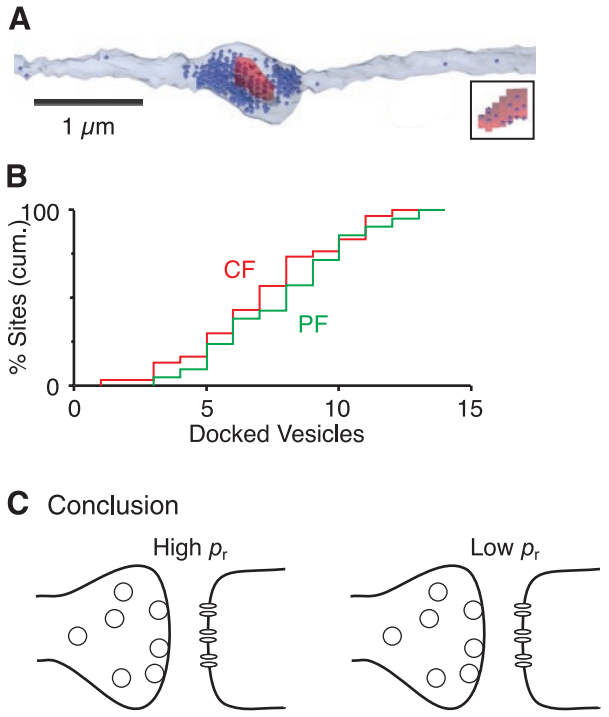

FIG. 6. Evidence that the number of docked vesicles does not determine the difference between P_r at climbing fiber and parallel fiber synapses. *A*: three-dimensional reconstruction of a portion of parallel fiber axon (light blue), with one release site (red), and all vesicles (dark blue). *Inset*: face-on views of a release site showing only docked vesicles (dark blue) across from the PSD (red). *B*: number of docked vesicles at synapses made by climbing fibers (red) and parallel fibers (green). Data are presented as a cumulative histogram. For climbing fiber reconstructions, see Figure 2. *C*: conclusion from these data that high and low P_r synapses have the same number of docked vesicles. [*A* and *B* adapted from Xu-Friedman et al. (131).]

ability of release or the accompanying differences in short-term plasticity (Fig. 6*C*). This is perhaps not surprising when one considers the many additional factors suggested above that could influence the probability of release.

IV. DEPLETION

Depletion has been proposed to account for depression at some synapses (9, 42) (Fig. 1*B*). To establish depletion as a reasonable model for depression requires examination of a set of physiological and anatomical parameters. *1*) The amount of depression needs to be measured physiologically, for example, by stimulating the presynaptic terminal with pairs of closely spaced action potentials and recording currents in the postsynaptic cell. *2*) It must be established that a component of depression is presynaptic in origin, rather than due to postsynaptic mechanisms such as desensitization. *3*) The number of vesicles released per site must be estimated using physiological methods. *4*) The number of vesicles available for release must be measured using physiological or anatomical techniques.

One synapse that has been examined for each of these criteria is the cerebellar climbing fiber. The climbing fiber shows strong depression, such that the second PSC is ~40% of the first at short intervals (67, 90). The source of this depression appears to be presynaptic, as desensitization does not contribute (34, 56). In addition, as described in section II*A*, it has been estimated that three or four vesicles per site are released on the first action potential (48, 129). Finally, serial reconstructions of climbing fiber release sites have found an average of seven docked vesicles (131). The release of three or four vesicles from a pool of seven docked vesicles could be reasonably expected to deplete release sites so that the response by the second pulse is diminished. Thus the synthesis of this information suggests that depletion is likely to contribute to depression at the climbing fiber.

How well does the depletion model apply to other synapses? Few synapses have been examined for all the criteria listed above, but some conclusions can be drawn from the partial information that does exist. The number of docked vesicles per release site has been documented for several types of synapses and ranges from 2 to 30. Depletion may therefore arise under considerably different circumstances for different synapses. For example, the calyx of Held has two or three docked vesicles per release site (104, 117). Therefore, release of even a single vesicle per site would be sufficient to cause significant depletion. In contrast, layer 1b to pyriform cortical pyramidal synapses have 27 docked vesicles on average (106). Therefore, depletion would require either considerable multivesicular release or a long train of stimuli before a significant number of these vesicles could be depleted.

V. POSTSYNAPTIC CONTRIBUTIONS TO SHORT-TERM SYNAPTIC PLASTICITY

There are at least two postsynaptic phenomena that can also affect short-term synaptic plasticity: saturation and desensitization. Both these phenomena seem to vary considerably at different types of synapses. One problem in determining the mechanisms underlying saturation and desensitization is that both depend critically on several factors that are difficult to measure: the time course of neurotransmitter within the synaptic cleft and the detailed kinetics and density of the postsynaptic receptors.

The importance of these becomes clear if we consider what happens to the transmitter that is released from a site. Transmitter rapidly diffuses away from the release site (see, for example, Fig. 4). In addition, transmitter transporters may be located on glia and neurons (13, 26, 99), which can affect the time course of transmitter in the synaptic cleft (6, 30). For synapses without glia, and where the pre- and postsynaptic neurons do not express transporters, transmitter would be cleared mainly

by diffusion (Fig. 7). At these synapses, blocking of transporters has no effect on an individual PSC (31, 87). The rate at which transmitter is cleared can affect whether receptors at the release site become saturated or desensitized.

Furthermore, the diffusing transmitter may affect other neurons as it escapes the synaptic cleft. At some synapses, release sites are physically shielded by barriers to diffusion such as intervening neural or glial processes (Fig. 7, isolated). However, others are not, which means that the transmitter released from one site has the potential to affect nearby release sites (Fig. 7, spillover). Spillover of transmitter into adjacent release sites can lead to activation, saturation, or desensitization at distant release sites. The importance of spillover has been the subject of much debate, but there appears to be increasing evidence that it can happen and that it does affect short-term synaptic plasticity (14, 121, 132).

A. Postsynaptic Receptor Saturation and Short-Term Plasticity

We have already mentioned postsynaptic receptor saturation as a complicating factor in determining P_r, the number of vesicles released, and quantal variability. More thorough reviews of the general issue of saturation are found elsewhere (51). Here we focus on recent evidence for the effects of saturation on short-term plasticity.

Saturation can cause synaptic depression at synapses that contain receptors with slow kinetics. For example, at some synapses when a release site is stimulated with two closely spaced action potentials, the NMDA receptors may be partially saturated after the first action potential, such that most receptors are still bound to glutamate when the second action potential arrives (32). Thus the amplitude of the second NMDA PSC will be much smaller than the first. One approach to verifying that this depression is independent of a presynaptic drop in glutamate release is by monitoring the AMPA PSC, which in some cases is less sensitive to saturation (32). A second approach to demonstrating NMDA receptor saturation is to use low-affinity NMDA receptor antagonists, which reduce saturation. An example of this is found at the retinogeniculate synapse, where the low-affinity antagonist L-(+)-2-amino-5-phosphonopentanoic acid (L-AP5) also reduced the amount of paired-pulse depression (14).

However, for synapses that undergo multivesicular release and depletion, saturation can have the opposite effect and reduce synaptic depression. The best illustration comes from work at the cerebellar climbing fiber. As described above, when pairs of pulses are applied to the climbing fiber, application of the low-affinity AMPA receptor antagonist DGG increased the amount of depression (48, 129). This appears to happen because, in control conditions, when multiple vesicles per site are released on the first pulse, postsynaptic receptors are significantly saturated, so the response is smaller than if the same number of vesicles were released at independent, unsaturated sites. However, the second pulse triggers release of fewer vesicles per site, so the postsynaptic response reflects the amount of presynaptic release more faithfully. Thus saturation reduces the effects of depletion. This same effect has also been found by using two drugs that reduce vesicular glutamate concentrations, bafilomycin A_1 and concanamycin A (55). These drugs reduce saturation by reducing the amount of glutamate released per vesicle, and also increase depression.

In addition, saturation can accelerate recovery from depression (48). For AMPA receptor-mediated PSCs at the climbing fiber, the rate of recovery is slowed by a factor of two in the presence of DGG. This appears to happen because under control conditions where there is saturation, each release site need only recover part of the releasable pool that is present under resting conditions to be release competent. In contrast, when postsynaptic receptor saturation is reduced by a low-affinity antagonist, each release site must restore its entire releasable pool.

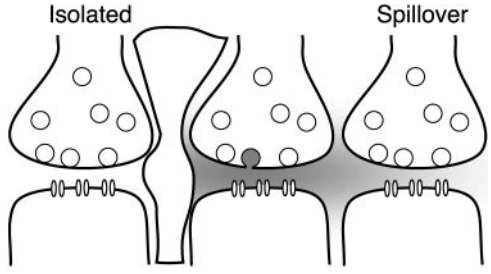

FIG. 7. Spillover from release sites. Glutamate diffuses away from a release site (*middle*). Barriers to diffusion, such as glia, may prevent its interacting with some release sites so that they are isolated. Other release sites lack barriers to diffusion and may be affected by diffusing glutamate through spillover.

B. Desensitization

Receptor desensitization is a second postsynaptic mechanism that contributes to short-term plasticity (119). Desensitization affects all ionotropic receptors. However, the sensitivity and time course vary considerably (63). For example, AMPA receptors composed of $GluR4_{flop}$ subunits desensitize after ~1 ms of glutamate exposure, whereas NMDA receptors desensitize on the time scale of hundreds of milliseconds (32). Thus the amount of desensitization expressed by different synapses depends on the kinetics of transmitter in the synaptic cleft and on the receptor kinetics.

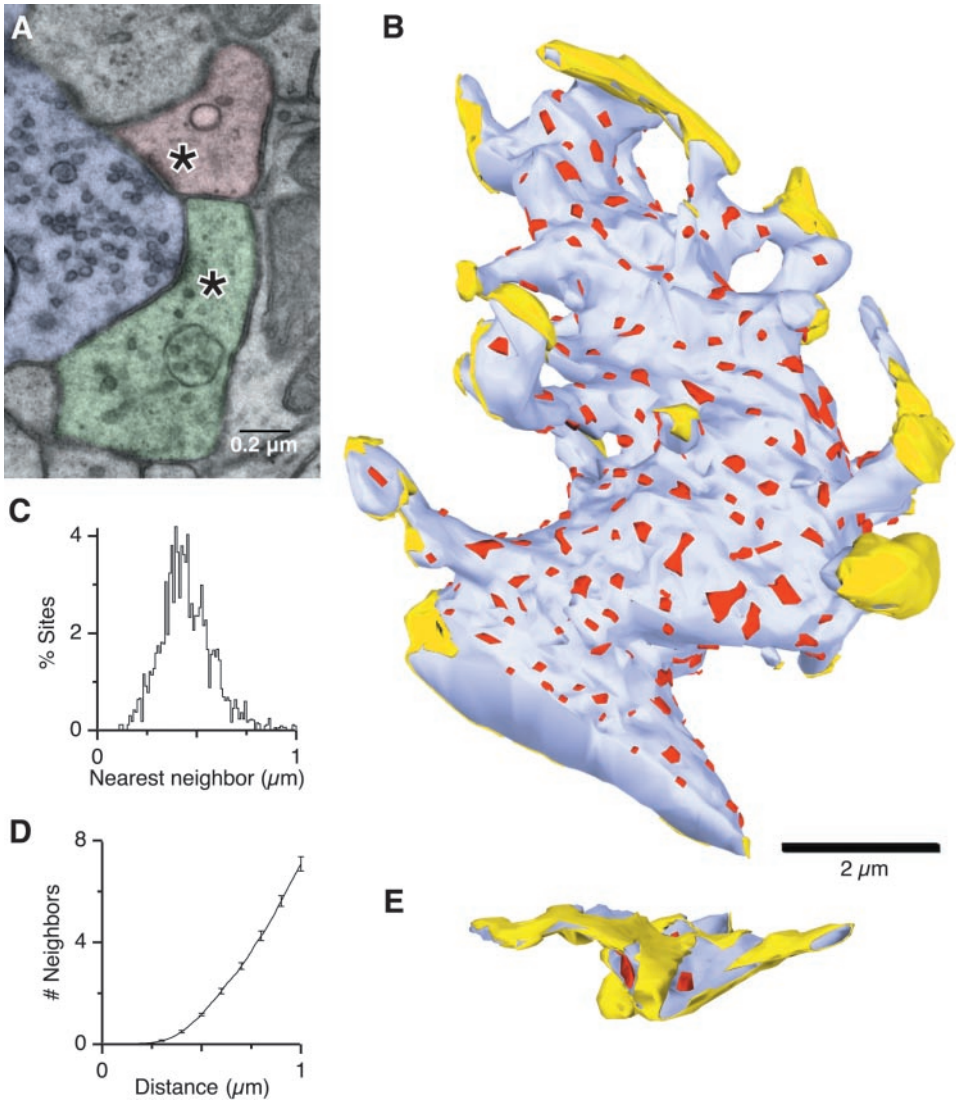

FIG. 8. Release site spacing at the cerebellar mossy fiber. *A*: electron micrograph of two release sites made by a mossy fiber (blue) onto two separate granule cell dendrites (pink, green). Both release sites (asterisks) have a PSD, widening of the synaptic cleft, active zone material, and a cluster of vesicles, some of which appear docked. *B*: three-dimensional reconstruction of a mossy fiber. The mossy fiber axon (blue) makes hundreds of synaptic contacts onto granule cell dendrites (red). Mossy fiber membrane contacts glia (yellow) only over ~20% of its surface, and typically out on small processes that do not contain many release sites. *C*: distances between nearest neighbors. The mean distance was ~0.5 μm. *D*: number of neighbors near release sites. On average, each release site has 7 neighbors within 1 μm. *E*: segment of a climbing fiber (same as in Fig. 2*C*) showing extensive coverage by glia, including between release sites. [Adapted from Xu-Friedman et al. (131).]

Desensitization appears to play more of a role at some synapses than others. This issue is most easily studied at AMPA-type glutamate receptors, because there are several drugs that interfere with desensitization, including cyclothiazide and aniracetam (128, 133). There are several synapses where application of these drugs reduced the amount of depression, including the avian and mammalian auditory brain stem (60, 121), the retinogeniculate synapse (14), the layer II/III cortical pyramidal to bipolar synapse (101), and the cerebellar mossy fiber to granule cell synapse (132). However, there are synapses where cyclothiazide has no effect on depression, including the cerebellar climbing fiber to Purkinje cell synapse (34, 56), the chick magnocellularis to laminaris synapse (69), and hippocampal CA3 to CA1 synapses (24).

To understand why some synapses are more subject to desensitization than others, one approach is to consider the structural differences between them. Among the synapses whose structure has been well characterized, those most subject to desensitization have distinctive glomerular or calyceal structures, whereas those least subject to desensitization are in bouton-like synapses (52, 62, 81, 89, 132). There are two key factors that differ between these types of synapses. First, desensitizing synapses have many release sites close together. Second, the release sites are not well isolated from each other by intervening glia. For example, cerebellar mossy fibers end in glomerular terminals onto granule cell dendrites (Fig. 8). They show depression, part of which appears to be due to desensitization (132). Furthermore, the release sites are located very close to each other with little intervening glia (Fig. 8). In contrast, climbing fiber to Purkinje cell synapses do not show any desensitization-dependent depression (34, 56). In addition, they are well surrounded by glia (Figs. 2*A* and 8*E*).

How do these anatomical features of close spacing

between release sites and a lack of glial ensheathment lead to desensitization? Glia may serve as physical barriers to diffusion. In addition, glia are known to express transporters for neurotransmitters (13, 99) and may thus contribute to rapid transmitter clearance from the synaptic cleft. Therefore, synapses that lack glia may be more susceptible to spillover. The importance of spillover to desensitization was first described at the calyceal synapse onto chick magnocellularis neurons (121). The calyx is a very large synapse made by a single axon with many release sites that are not insulated from each other by glia. Chick magnocellularis neurons express rapidly desensitizing AMPA receptors, which are highly sensitive to glutamate exposure (92). Under high P_r conditions, desensitization makes a large contribution to paired-pulse plasticity (Fig. 1*C*). It was proposed that glutamate released from multiple release sites pools and prolongs receptor exposure (121) (Fig. 9). Thus a large fraction of receptors enters a desensitized state, reducing the amplitude of the second EPSC beyond the amount caused by presynaptic depletion. At low P_r, however, no desensitization was seen, which suggests that pooling from multiple release sites was critical to induce desensitization.

However, anatomical data have suggested that desensitization could be important even when P_r is low, provided release site spacing is very close (Fig. 9). Release site spacing in a desensitizing synapse has been measured most carefully at the cerebellar mossy fiber. The mossy fiber axon forms a large terminal with hundreds of release sites onto the dendrites of many granule cells (62). Serial electron microscopic reconstructions showed that nearest neighboring release sites are ~0.5 μm apart on average (center to center), and the number of neighboring release sites within 1 μm is ~7 (132) (Fig. 8, *C* and *D*).

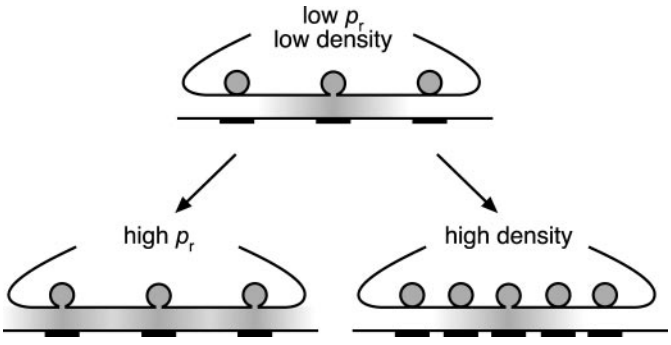

FIG. 9. Two anatomical arrangements that give rise to desensitization. *Top*: schematic of a low P_r synapse with low release site density. Three release sites are symbolized by docked vesicles in the presynaptic axon across from PSDs. Release from one site produces insufficient spillover to induce desensitization. *Bottom left*: a high P_r synapse. Under these conditions, glutamate released from many sites pools to produce a large, prolonged glutamate signal, which is sufficient to cause desensitization. *Bottom right*: a synapse with high release site density. Release from a single site induces desensitization in its nearest neighbors because they are so close. [Modified from Trussell et al. (121).]

Physiological studies have provided evidence that these release sites are sufficiently close for spillover to take place and activate AMPA receptors at adjacent release sites (31). This raises the possibility that receptors at adjacent release sites could also be desensitized, particularly as granule cells also express the rapidly desensitizing $GluR4_{flop}$ (79). This is unlike the climbing fiber where release sites are isolated by glia and glutamate transporters (26, 86, 131) (Fig. 8*E*). Modeling of glutamate diffusion and AMPA receptor desensitization at the mossy fiber suggests that, at 1 μm away and 10 ms after release of a single vesicle, 20–60% of AMPA receptors may be desensitized, depending on the AMPA receptor kinetics (132). Thus a single vesicle released from a single site could significantly affect receptor availability at as many as seven neighboring release sites. Similarly, a single release site could be significantly affected if any of its seven neighbors releases a vesicle, which is highly likely even when P_r is <0.2. Thus the mossy fiber glomerular structure with its high density of release sites would allow desensitization to play a role in short-term plasticity even for a low P_r synapse.

VI. CONCLUSION

Analysis of the ultrastructure of synapses provides a useful tool for understanding the mechanisms underlying synaptic plasticity. It is also important that it be used in conjunction with physiology. The current evidence suggests that the balance of facilitation and depression depends on P_r. While the number of docked vesicles has been proposed to contribute to P_r at one class of synapse, it is clearly not the primary determinant of P_r at other synapses. Depletion of the docked vesicle pool can account for depression at some synapses, although its importance depends on the amount of multivesicular release and postsynaptic receptor saturation. Postsynaptic features such as saturation and desensitization also play a role in the amount of depression and the rate of recovery. Saturation may depend on the size of the postsynaptic density, which varies for different synapses. The amount of desensitization appears to depend on the amount of spillover between release sites, which is affected by the presence of glia that take up transmitter and by the spacing of release sites.

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