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Are Unreliable Release Mechanisms Conserved from NMJ to CNS?

Tyler B. Tarr^{1,2}, Markus Dittrich^{1,3,4}, and Stephen D. Meriney^{1,2}

¹Department of Neuroscience, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

²Center for the Neural Basis of Cognition, Pittsburgh, PA 15213

³National Resource for Biomedical Supercomputing, Pittsburgh Supercomputing Center, Pittsburgh, PA 15213

⁴Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA, 15260

Abstract

The frog neuromuscular junction (NMJ) is a strong and reliable synapse because during activation sufficient neurotransmitter is released to trigger a postsynaptic action potential. Recent evidence supports the hypothesis that this reliability emerges from the assembly of thousands of unreliable single vesicle release sites. The mechanisms that govern this unreliability include a paucity of voltage-gated calcium channels, a low probability of calcium channel opening during an action potential, and the rare triggering of synaptic vesicle fusion even when a calcium channel does open and allow calcium flux. Here we discuss the evidence that these unreliable single vesicle release sites may be the fundamental building blocks of many types of synapses in both the peripheral and central nervous system.

Keywords

Calcium; Neurotransmitter release; Synapse

Introduction

Neurotransmitter release at synapses is known to underlie most functions throughout the nervous system. Release of neurotransmitter typically occurs within specialized regions of the nerve terminal called active zones, which are highly organized for synaptic vesicle docking and fusion [1] (Glossary). The basic hypothesis of calcium (Ca^{2+})-triggered synaptic vesicle fusion was first proposed based on work in the frog NMJ, and has since

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Corresponding author: Meriney, SD (meriney@pitt.edu).

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been demonstrated to be conserved across many synapses and species [2,3]. Even though we have known for more than 60 years that Ca^{2+} ion flux into the presynaptic nerve terminal triggers chemical transmitter release, many details of this process remain unknown. This is largely due to the difficulty of experimentally accessing the small sub-compartment within neurons where active zones are located.

Historical perspective on presynaptic Ca^{2+} entry and transmitter release

The proposed mechanism for unreliable vesicle release which we put forth in this opinion piece builds upon a number of previous observations in several different model systems (see [4] for review). For example, using the squid giant synapse, a combination of imaging and electrophysiological techniques were used to identify an action potential (AP)-triggered, spatially localized domain of elevated Ca^{2+} near the mouth of open Ca^{2+} channels [5]. Calcium-dependent triggering of vesicle release was also examined in the central nervous system (CNS) within the immature calyx of Held synapse in the auditory brainstem and shown to be controlled by the summed Ca^{2+} signal that resulted when many Ca^{2+} channels opened simultaneously in the nerve terminal (microdomain coupling; Box 1; [6, 7]). Similar results were obtained when cerebellar climbing fiber and parallel fiber synapses were examined [8, 9]. These cerebellar synapses appeared to reliably drive postsynaptic cells only via activation of a large fraction of available presynaptic Ca^{2+} channels during an AP stimulation [9], and were shown to have a Ca^{2+} channel-release site relationship of about four [8] (Box 2). Based on these findings in the immature calyx of Held and cerebellar synapses, and subsequent generalizations to other systems, synaptic vesicle release was hypothesized to be chiefly controlled by microdomain coupling (Box 1).

The experiments described above generally over-shadowed the few early reports that supported the hypothesis that transmitter release at synapses could be triggered by the Ca^{2+} flux through a single open Ca^{2+} channel (nanodomain coupling; Box 1). For example, studies of the chick ciliary ganglion calyx synapse showed that Ca^{2+} flux through a single open Ca^{2+} channel could trigger a vesicle fusion event [10], and that these synapses had a Ca^{2+} channel-release site relationship close to 1 [11] (Box 2). Therefore, although transmitter release at chick calyx synapse was hypothesized to be triggered by nanodomain coupling, the general applicability of this mechanism for triggering transmitter release at other synapses was not immediately embraced. However, recent studies which have shown that nanodomain coupling may be operative in a variety of other systems warrant a close re-examination of this mechanism. In fact, as we discuss below, this type of coupling between Ca^{2+} channels and synaptic vesicles constitutes an essential component of our unreliable vesicle release hypothesis.

The unreliable single vesicle release site as a basic building block of synapses

Recent advances in preparation of adult neural tissue for live cell study, electrophysiological recording, cellular imaging, and computer modeling have increased the number of synapses where studies of sub-active zone mechanisms have been performed. This has provided additional insight into the coupling of Ca^{2+} flux to synaptic vesicle release and enabled the

re-examination of some of the original concepts. Based on recent studies from several model systems, we hypothesize that many synapses in both the peripheral nervous system (PNS) and CNS are built using a similar basic building block: the unreliable single vesicle release site (Figure 1a). These unreliable single vesicle release sites consist of a plasma membrane-associated synaptic vesicle, its fusion machinery, and a sparse number of closely associated Ca^{2+} channels that each open with low probability during an AP stimulus and trigger transmitter release using nanodomain coupling. Here, the term *unreliable* makes explicit that an AP stimulus will trigger release of any particular vesicle with low probability. The overall properties of each synapse are then partly determined by the way in which these single vesicle release sites are assembled, first into active zones, and then into complete synapses. To support our hypothesis, we have focused on data from model preparations where sufficient relevant experimental data are available, with particular emphasis on studies that address Ca^{2+} -dependent transmitter release mechanisms at single vesicle release sites.

The frog NMJ as a model synapse

The adult frog NMJ is a classic model synapse that has played a critical role in our understanding of the basic mechanisms of synaptic transmission [2]. The frog NMJ is a strong and reliable synapse in the sense that a single presynaptic AP leads to the release of ~350 quanta from the entire nerve terminal [12]. This magnitude of transmitter release is more than sufficient to bring the postsynaptic muscle cell to threshold, resulting in reliable muscle contraction [13]. Ultrastructurally, the frog NMJ contains an average of 700 linear (1 μm long) active zones separated from one another by about 1 μm [12, 14]. Freeze fracture and electron microscope tomography have provided detailed structural information demonstrating that these active zones each contain ~30–40 docked synaptic vesicles arranged in a double row along the length of this structure, each tightly associated with vesicle release machinery and “ready” for Ca^{2+} -triggered fusion [15–18]. Based on the number of released quanta and the number of active zones per synapse, each active zone releases on average a single vesicle every other AP stimulus (probability of release per active zone = 0.5). Thus, the probability of release for any single synaptic vesicle is very small. This unreliable release within each active zone ensures that the NMJ retains a large fraction of its available docked vesicles (a conservation of resources), even during repeated stimulation, resulting in strong and reliable release from the whole NMJ during sustained activity.

Frog NMJ active zones also contain ~200–250 intramembranous particles (some of which are thought to be Ca^{2+} channels) arranged in two parallel rows between the double row of vesicles [14–16]. Combining high-resolution Ca^{2+} imaging techniques with variance analysis of imaged Ca^{2+} signals has enabled the study of voltage-gated Ca^{2+} channel properties in small neuronal compartments inaccessible to standard electrophysiological techniques [19]. This method has previously revealed the stochastic opening of a small number of Ca^{2+} channels in both presynaptic boutons and postsynaptic spines [19, 20]. Initial work demonstrated the feasibility of imaging presynaptic Ca^{2+} entry at frog NMJ active zones [21]. A subsequent study used single pixel optical fluctuation analysis (SPOFA) of imaged AP-evoked Ca^{2+} fluorescence at the frog NMJ to estimate the number and

opening probability of Ca^{2+} channels during an AP at single active zones [22]. This work identified the mechanisms that govern the low probability of transmitter release at active zones in the frog NMJ, which include a paucity of voltage-gated Ca^{2+} channels (30–40 per active zone, which represents only about 15–20% of total intramembranous particles identified in freeze fracture studies), a low probability of Ca^{2+} channel opening during an AP stimulus (0.2 – 0.3), and the rare triggering of synaptic vesicle fusion when a Ca^{2+} channel does open and allow local Ca^{2+} flux [22]. Interestingly, these data predict that the total number of presynaptic Ca^{2+} channels within an individual active zone coincides roughly with the number of docked synaptic vesicles and that during an AP only ~6–10 Ca^{2+} channels open in each active zone. This quantitative relationship suggests that each single vesicle release site in the frog NMJ associates on average with only a single voltage-gated Ca^{2+} channel, and that vesicle fusion is triggered by the nanodomain of relatively high Ca^{2+} concentration near the mouth of a single open Ca^{2+} channel.

These findings are also consistent with previous studies at this synapse that have proposed that Ca^{2+} flux through only one or two Ca^{2+} channels can trigger transmitter release [23, 24]. Experiments combining high resolution Ca^{2+} imaging [22] and MCell [25] computer modeling have provided evidence that each vesicle fusion event at this synapse is controlled predominately by a single Ca^{2+} channel positioned nearby. Further, it appears that Ca^{2+} flux through this nearby channel triggers vesicle fusion with a low probability (5~10%) [22].

Together these results suggest that each individual docked vesicle, its release machinery, and a single (or small number of) tightly associated voltage gated Ca^{2+} channel(s), forms a basic unit responsible for low probability vesicle release. We call this unit an unreliable single vesicle release site (Figure 1a) since vesicle release from an individual site in response to an AP is rare. The overall release properties of the frog NMJ active zone then emerge as the sum of 30–40 such low probability single vesicle release sites, and the overall release properties of the entire NMJ emerge as the sum of the activity of hundreds of such active zones.

Are similar presynaptic mechanisms used at the mammalian NMJ?

Compared to the frog NMJ, the mammalian NMJ has a different active zone organizational structure (Figure 1). Specifically, the mouse NMJ consists of many swellings or boutons, each with an area of ~5–10 μm^2 that contain about 15–20 active zones [26]. At these mammalian NMJs, each active zone is about 100–200 nm long (separated from one another by about 500 nm [27]), contains about 20 intramembranous particles arranged in two double rows, and 2–3 vesicles docked between these particle rows [28]. Assuming the same percentage of Ca^{2+} channels among intramembranous particles at the mouse NMJ as in the frog, there may only be 4–5 functional Ca^{2+} channels per active zone. Since synaptic vesicles run down the middle of each mammalian active zone, each vesicle may be closely associated with 2–3 Ca^{2+} channels bilaterally.

How do these structural differences between mouse and frog active zones correlate with observed electrophysiological measurements, and what does this tell us about the underlying functional units responsible for release? At the frog NMJ, a single AP triggers the release of

~350 quanta from a total of ~700 active zones. Assuming ~30–40 docked vesicles at each frog NMJ active zone, the probability of release for any given vesicle is 1–2%. At a typical mouse NMJ, there are a similar number of active zones (~600–800), but quantal content is only ~60–80 [27, 29]. Assuming two vesicles per mammalian active zone, the release probability per vesicle is 5% and thus slightly higher than in the frog. This increase, however, may simply result from the increased number of Ca^{2+} channels that are closely associated with each synaptic vesicle (two versus one at the frog, neglecting potential gating differences between N-type Ca^{2+} channels at frog NMJs, and P/Q-type Ca^{2+} channels at mammalian NMJs). Thus, it appears that unreliable single vesicle release sites in the mouse and frog (each consisting of a docked vesicle, release machinery, and its closely associated Ca^{2+} channels) are very similar. We hypothesize that differences in the release properties of their active zones and NMJs (including short-term plasticity effects) are to a significant degree a function of how many single vesicle release sites are present and their spatial and stoichiometric relationship with one another.

To further support this hypothesis at the mammalian NMJ, it would be interesting to perform Ca^{2+} imaging experiments similar to the ones described for the frog NMJ [22] to estimate the number of Ca^{2+} channels per active zone, their opening probability during an AP, and the number of Ca^{2+} channels that contribute Ca^{2+} ions to each vesicle release event.

Are unreliable single vesicle release sites also used at CNS synapses?

Several CNS synapses share similar requirements of reliability and strength with the NMJ. One such system that has been studied extensively is the calyx of Held synapse, which has become a popular model system due to the accessibility of its presynaptic terminal to direct electrophysiological manipulation [30, 31]. Studies of the mature rat calyx of Held (~P14 or older, which is considered mature for this synapse as it corresponds to the onset of hearing [31]) have shown that the synapse has about 700 active zones, each with an average of three docked synaptic vesicles [32]. The probability of vesicle fusion at each of these single vesicle release sites appears to be very low (~0.13) [32]. Additionally, the Ca^{2+} channel-release site relationship (Box 2) follows a power law with an exponent of less than 3 [33, 34], indicating that only a small number of channels contribute to release. Finally, it has been shown that the mature calyx of Held has a very fast AP waveform, leading to the recruitment of only a small fraction of Ca^{2+} channels upon AP invasion into the terminal [35, 36]. These findings demonstrate that the mechanisms that govern synaptic vesicle release at the calyx of Held are similar to those found at the NMJ, namely single vesicle release sites with a low vesicle release probability triggered by Ca^{2+} from a small number of closely-associated open Ca^{2+} channels.

Interestingly, this situation is quite different from what emerged based on earlier studies performed in slice preparations of the immature calyx of Held (~P10 or younger). These immature calyx synapses have roughly the same number of total docked vesicles as their mature counterparts but only about half as many active zones, and thus, about twice as many docked vesicles per active zone as the mature calyx [32, 37]. Patch-clamp recordings of Ca^{2+} current showed that a single AP-like waveform could activate a Ca^{2+} current that is about 70% of the maximum achievable with a prolonged waveform [6]. Furthermore,

experiments to determine the Ca^{2+} channel-release site relationship found a power relationship with an exponent of about 3–5 [7, 33], indicating that Ca^{2+} flux through many Ca^{2+} channels triggers the release of a single vesicle (Figure 1b). In support of this conclusion, experiments show that the slow Ca^{2+} buffer EGTA has similar effects on release as the fast buffer BAPTA [33, 38]. Thus, it appears that as these synapses mature there is a transition from microdomain coupling in immature synapses to unreliable single vesicle release sites controlled by nanodomain coupling in adult synapses. Further, narrowing of the AP during development and maturation at this synapse may attenuate calcium influx, but the switch from microdomain to nanodomain coupling may effectively boost synaptic efficacy and counteract the AP narrowing effect [39].

Studies at other large CNS synapses have also provided results that are reminiscent of the mechanisms employed at the NMJ. One such synapse is the dentate gyrus basket cell-granule cell (BC-GC) synapse, which is a synapse known for high temporal fidelity and reliable spiking [40]. This synapse is estimated to contain three to seven active zones, and each active zone appears to have a release probability of ~0.5 and an estimated releasable vesicle pool size at each active zone of around 50 [41]. Thus, the probability of release per vesicle is very low. Ultrastructural analysis and modeling data show a very short distance between the Ca^{2+} channels and the Ca^{2+} sensor (~10–20 nm) [42], suggesting nanodomain coupling between presynaptic Ca^{2+} channels and vesicle release sites. This is supported further by experiments with exogenous chelators, which show that release is inhibited by the fast Ca^{2+} chelator BAPTA, but not by the slow Ca^{2+} chelator EGTA [42]. Finally, determination of the Ca^{2+} channel-release site relationship, coupled with computer modeling, indicate that only a few Ca^{2+} channels are involved in the release of each vesicle [43]. With its large pool of releasable vesicles, a low probability of release per vesicle, and tight coupling between Ca^{2+} channels and vesicle release sites, the reliable BC-GC synapse appears to be built from unreliable single vesicle release sites in a fashion similar to the NMJ.

Another large, multi-active zone synapse that has been studied extensively is the hippocampal mossy fiber synapse between dentate gyrus granule cells and CA3 pyramidal cells. This is a large synapse known for high temporal fidelity and reliable spiking [44], and is often used in studies of synaptic plasticity [45–47]. The mossy fiber synapse is characterized by an average of about 30 active zones [48–50], and with an estimated readily releasable pool of about 1400 vesicles [51], each active zone has ~46 single vesicle release sites. Under low frequency stimulation conditions, the release probability per active zone is quite low (<0.3) [52]. Consequently, the release probability per vesicle is substantially lower. However, in contrast to the NMJ, there appears to be a large number of presynaptic Ca^{2+} channels at the mossy fiber synapse [53], and these Ca^{2+} channels appear to be efficiently activated by a single AP [54]; AP waveforms were shown to open ~80% of the maximal current that could be activated with a long square depolarization [54]. Thus, Ca^{2+} channels open with a high probability during an AP, and the flux through many Ca^{2+} channels may be required to trigger the release of a single vesicle. This is reminiscent of the situation in the immature calyx of Held. In fact, the hippocampal mossy fiber synapse studied in acute hippocampal slices is often removed for study from relatively young rats, and may indeed be a relatively immature synapse. The synapse itself forms along the axon of the dentate granule neuron, which is one of only a few neurons in the CNS that continues

to be born in adult animals from a proliferation zone near the dentate gyrus [55–57]. The dentate granule cells are likely to be constantly turning over and reforming mossy fiber synapses [58], perhaps in response to plasticity associated with learning and memory [59, 60]. Therefore, it is possible that even in the adult animal this synapse retains some immature features (including the organization of single vesicle release sites). We will have to await further studies to gain additional insight into this issue.

Conclusions

Based on the available data from synapses of both the PNS and CNS, we suggest that a functionally heterogeneous population of synapses can be built via assembly and spatial organization of varying numbers of a basic unit: the low probability (unreliable) single vesicle release site. In this view, smaller unreliable synapses are characterized by only a few unreliable single vesicle release sites whereas the reliability of larger synapses derives from assembling a large number of them. Beyond that, the spatial organization of single vesicle release sites in individual active zones offers even more functional diversity. Therefore, one way in which synapses can be built to be reliable or unreliable is by varying the number and spatial organization of these unreliable building blocks.

Why should the fundamental unit of transmitter release be unreliable? While the answer to this question is not yet fully understood, several hypotheses have been advanced. At small CNS synapses that only contain one or a few active zones, only a small total number of unreliable single vesicle release sites will be present, rendering the synapses unreliable. Small CNS synapses have been reported to be highly unreliable for many years (see [61] for example), although the magnitude of unreliability may be temperature dependent [62]. At CNS synapses that often fire in bursts, unreliable synapses offer a broader dynamic range of transmitter release and do not result in vesicle depletion during normal activity patterns, which are properties important for information processing in the brain [63]. Similarly, the use of unreliable single vesicle release sites within large synapses that may contain hundreds or thousands of these units creates synapses that on the whole release enough transmitter to reliably activate postsynaptic cells. Yet, these synapses do not fatigue with continued or tonic use because only a small percentage of the available release sites are used with each AP. This conservation of resources may be a major reason why NMJs do not show significant short-term depression during short trains of AP activity – an important quality that underlies function at this synapse. Thus, as a basic unit, an unreliable release site provides significantly more flexibility than a high fidelity site in assembling synapses with a large range of properties and characteristics.

The assembly of unreliable single vesicle release sites in active zones could be accomplished via active zone precursor vesicles (or small dense core vesicles) that contain a collection of pre-assembled presynaptic plasma membrane-associated active zone proteins, as have been shown to exist in developing neurons *in vitro* [64, 65]. After these release site components are inserted into the active zone region of the nerve terminal, Ca^{2+} channels may associate with them in a manner carefully regulated by intracellular biochemical events, which control whether microdomain or nanodomain coupling to vesicle release will occur. Differential regulation of Ca^{2+} channel coupling to vesicle release sites as synapses mature may then

explain why some immature synapses appear to use microdomain coupling, while mature synapses often use nanodomain coupling (at some synapses, like cultured frog NMJs, nanodomain coupling may be present even at early developmental stages; [66, 67]). The development of nanodomain coupling at mature vesicle release sites in the CNS may involve alterations in protein-protein interactions between release site proteins and Ca^{2+} channels (see for example [68, 69]) and/or changes in the phosphorylation state of some of these proteins, which may modulate the probability of Ca^{2+} channel opening during an AP (see for example [70–75]). In this way, active zones initially assembled in a microdomain fashion could gradually reduce the number of vesicle associated Ca^{2+} channels during development, morphing into low probability single vesicle release sites with nanodomain coupling once mature. Consistent with this hypothesis, there is evidence at some synapses that the probability of release decreases as synapses mature [32, 35, 76–79].

Although our hypothesized low probability single vesicle release site mechanism for regulating transmitter release may not act at all synapses, integration of numerous findings over the past few years suggests it as a fundamental principle of synapse assembly in a number of well-studied synapses. This realization has spawned a number of interesting and challenging new research questions (Box 3) that will prompt investigators to strive to elucidate additional details of the presynaptic mechanisms that control chemical transmitter release.

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glossary

Active zone	presynaptic area of a synapse containing a spatially distinct collection of single vesicle release sites
BAPTA	1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid an exogenous Ca^{2+} buffer with relatively fast binding kinetics that can interfere with Ca^{2+} triggered vesicle fusion even when Ca^{2+} channels are very tightly coupled to synaptic vesicles
Docked vesicles	neurotransmitter filled synaptic vesicles that are tightly associated with the presynaptic membrane in an active zone via proteins that mediate vesicle fusion
EGTA	ethylene glycol tetraacetic acid an exogenous Ca^{2+} buffer with relatively slow binding kinetics that can only interfere with Ca^{2+} triggered vesicle fusion when there is loose spatial coupling between Ca^{2+} channels and synaptic vesicles

Intramembranous particles	membrane embedded structures identified in freeze-fracture electron micrographs that are presumed to represent transmembrane proteins
Mcell	Monte Carlo Cell. A stochastic reaction–diffusion simulation package for realistic computer modeling of cellular signaling within the complex 3-dimensional subcellular microenvironment in and around living cells (www.mcell.org) [25]
Molecular cooperativity of transmitter release	relationship between extracellular Ca^{2+} ion concentration and vesicle fusion. This relationship is typically steep such that small changes in Ca^{2+} ion concentration lead to large changes in transmitter release. This relationship has a slope of about 4 when plotted on a log scale
N- and P/Q-type calcium channels	two families of voltage-gated Ca^{2+} channels that are commonly present at synapses to regulate Ca^{2+} -triggered vesicle fusion and the release of transmitter
Quanta	packages (or fixed numbers) of transmitter that are released upon vesicle fusion. Because transmitter is thought to be packaged at a regulated concentration into vesicles of a similar size, multiples of this fixed amount are released
Single vesicle release site	site within an active zone at which a single synaptic vesicle can dock and associate with voltage-gated Ca^{2+} channels and other proteins that mediate vesicle fusion
Vesicle release machinery	proteins within the single vesicle release site that mediate vesicle fusion with plasma membrane

References

1. Zhai RG, Bellen HJ. The architecture of the active zone in the presynaptic nerve terminal. *Physiology* (Bethesda). 2004; 19:262–270. [PubMed: 15381754]
2. Katz, B. The Release of Neural Transmitter Substances. Liverpool: Liverpool University Press; 1969.
3. Stevens CF. Neurotransmitter release at central synapses. *Neuron*. 2003; 40:381–388. [PubMed: 14556715]
4. Schneggenburger R, Neher E. Presynaptic calcium and control of vesicle fusion. *Curr. Opin. Neurobiol.* 2005; 15:266–274. [PubMed: 15919191]
5. Llinás R, et al. The concept of calcium concentration microdomains in synaptic transmission. *Neuropharmacology*. 1995; 34:1443–1451. [PubMed: 8606792]
6. Borst JG, Sakmann B. Calcium current during a single action potential in a large presynaptic terminal of the rat brainstem. *J. Physiol.* 1998; 506:143–157. [PubMed: 9481678]
7. Borst JG, Sakmann B. Effect of changes in action potential shape on calcium currents and transmitter release in a calyx-type synapse of the rat auditory brainstem. *Trans. Philos. R. Soc. Lond. B Biol. Sci.* 1999; 354:347–355.
8. Foster KA, et al. Interaction of postsynaptic receptor saturation with presynaptic mechanisms produces a reliable synapse. *Neuron*. 2002; 36:1115–1126. [PubMed: 12495626]

9. Brenowitz SD, Regehr WG. Reliability and heterogeneity of calcium signaling at single presynaptic boutons of cerebellar granule cells. *J. Neurosci.* 2007; 27:7888–7898. [PubMed: 17652580]
10. Stanley EF. Single calcium channels and acetylcholine release at a presynaptic nerve terminal. *Neuron.* 1993; 11:1007–1011. [PubMed: 8274272]
11. Gentile L, Stanley EF. A unified model of presynaptic release site gating by calcium channel domains. *Eur. J. Neurosci.* 2005; 21:278–282. [PubMed: 15654866]
12. Katz B, Miledi R. Estimates of quantal content during ‘chemical potentiation’ of transmitter release. *Proc. R. Soc. Lond. B Biol. Sci.* 1979; 205:369–378. [PubMed: 41252]
13. Wood SJ, Slater CR. Safety factor at the neuromuscular junction. *Prog. Neurobiol.* 2001; 64:393–429. [PubMed: 11275359]
14. Heuser JE, et al. Functional changes in frog neuromuscular junctions studied with freeze-fracture. *J. Neurocytol.* 1974; 3:109–131. [PubMed: 4596345]
15. Heuser JE, et al. Synaptic vesicle exocytosis captured by quick freezing and correlated with quantal transmitter release. *J. Cell Biol.* 1979; 81:275–300. [PubMed: 38256]
16. Pawson PA, et al. Quantitative freeze-fracture analysis of the frog neuromuscular junction synapse--I. Naturally occurring variability in active zone structure. *J. Neurocytol.* 1998; 27:361–377. [PubMed: 9923981]
17. Harlow ML, et al. The architecture of active zone material at the frog's neuromuscular junction. *Nature.* 2001; 409:479–484. [PubMed: 11206537]
18. Rizzoli SO, Betz WJ. Synaptic vesicle pools. *Nat. Rev. Neurosci.* 2005; 6:57–69. [PubMed: 15611727]
19. Sabatini BL, Svoboda K. Analysis of calcium channels in single spines using optical fluctuation analysis. *Nature.* 2000; 408:589–593. [PubMed: 11117746]
20. Frenguelli BG, Malinow R. Fluctuations in intracellular calcium responses to action potentials in single en passage presynaptic boutons of layer V neurons in neocortical slices. *Learn. Mem.* 1996; 3:150–159. [PubMed: 10456085]
21. Wachman ES, et al. Spatial Distribution of Calcium Entry Evoked by Single Action Potentials within the Presynaptic Active Zone. *J. Neurosci.* 2004; 24:2877–2885. [PubMed: 15044526]
22. Luo F, et al. Single-pixel optical fluctuation analysis of calcium channel function in active zones of motor nerve terminals. *J. Neurosci.* 2011; 31:11268–11281. [PubMed: 21813687]
23. Yoshikami D, et al. The inhibitory effects of omega-conotoxins on Ca channels and synapses. *Ann. N. Y. Acad. Sci.* 1989; 560:230–248. [PubMed: 2545135]
24. Shahrezaei V, et al. Ca^{2+} from one or two channels controls fusion of a single vesicle at the frog neuromuscular junction. *J. Neurosci.* 2006; 26:13240–13249. [PubMed: 17182774]
25. Kerr RA, et al. Fast Monte Carlo Simulation Methods for Biological Reaction-Diffusion Systems in Solution and on Surfaces. *SIAM J. Sci. Comput.* 2008; 30:3126. [PubMed: 20151023]
26. Slater CR. Structural factors influencing the efficacy of neuromuscular transmission. *Ann. N. Y. Acad. Sci.* 2008; 1132:1–12. [PubMed: 18096848]
27. Ruiz R, et al. Active zones and the readily releasable pool of synaptic vesicles at the neuromuscular junction of the mouse. *J. Neurosci.* 2011; 31:2000–2008. [PubMed: 21307238]
28. Nagwaney S, et al. Macromolecular connections of active zone material to docked synaptic vesicles and presynaptic membrane at neuromuscular junctions of mouse. *J. Comp. Neurol.* 2009; 513:457–468. [PubMed: 19226520]
29. Wang X, et al. Activity-dependent regulation of the binomial parameters p and n at the mouse neuromuscular junction in vivo. *J. Neurophysiol.* 2010; 104:2352–2358. [PubMed: 20739593]
30. Forsythe ID. Direct patch recording from identified presynaptic terminals mediating glutamatergic EPSCs in the rat CNS, in vitro. *J. Physiol.* 1994; 479:381–387. [PubMed: 7837096]
31. Borst JG, et al. Pre- and postsynaptic whole-cell recordings in the medial nucleus of the trapezoid body of the rat. *J. Physiol.* 1995; 489:825–840. [PubMed: 8788946]
32. Taschenberger H, et al. Optimizing synaptic architecture and efficiency for high-frequency transmission. *Neuron.* 2002; 36:1127–1143. [PubMed: 12495627]
33. Fedchyshyn MJ, Wang LY. Developmental transformation of the release modality at the calyx of Held synapse. *J. Neurosci.* 2005; 25:4131–4140. [PubMed: 15843616]

34. Kochubey O, et al. Developmental regulation of the intracellular Ca^{2+} sensitivity of vesicle fusion and Ca^{2+} -secretion coupling at the rat calyx of Held. *J. Physiol.* 2009; 587:3009–3023. [PubMed: 19403608]
35. Taschenberger H, von Gersdorff H. Fine-tuning an auditory synapse for speed and fidelity: developmental changes in presynaptic waveform, EPSC kinetics, and synaptic plasticity. *J. Neurosci.* 2000; 20:9162–9173.
36. Yang YM, Wang LY. Amplitude and kinetics of action potential-evoked Ca^{2+} current and its efficacy in triggering transmitter release at the developing calyx of held synapse. *J. Neurosci.* 2006; 26:5698–5708. [PubMed: 16723526]
37. Satzler K, et al. Three-dimensional reconstruction of a calyx of Held and its postsynaptic principal neuron in the medial nucleus of the trapezoid body. *J. Neurosci.* 2002; 22:10567–10579. [PubMed: 12486149]
38. Meinrenken CJ, et al. Calcium secretion coupling at calyx of held governed by nonuniform channel-vesicle topography. *J. Neurosci.* 2002; 22:1648–1667. [PubMed: 11880495]
39. Wang LY, et al. Action potential evoked transmitter release in central synapses: insights from the developing calyx of Held. *Mol. Brain.* 2009; 2:36. [PubMed: 19939269]
40. Jonas P, et al. Interneuron Diversity series: Fast in, fast out—temporal and spatial signal processing in hippocampal interneurons. *Trends Neurosci.* 2004; 27:30–40. [PubMed: 14698608]
41. Kraushaar U, Jonas P. Efficacy and stability of quantal GABA release at a hippocampal interneuron-principal neuron synapse. *J. Neurosci.* 2000; 20:5594–5607. [PubMed: 10908596]
42. Bucurenciu I, et al. Nanodomain coupling between Ca^{2+} channels and Ca^{2+} sensors promotes fast and efficient transmitter release at a cortical GABAergic synapse. *Neuron.* 2008; 57:536–545. [PubMed: 18304483]
43. Bucurenciu I, et al. A small number of open Ca^{2+} channels trigger transmitter release at a central GABAergic synapse. *Nat. Neurosci.* 2010; 13:19–21. [PubMed: 20010820]
44. Henze DA, et al. Giant miniature EPSCs at the hippocampal mossy fiber to CA3 pyramidal cell synapse are monoquantal. *J. Neurophysiol.* 2002; 87:15–29. [PubMed: 11784726]
45. Henze DA, et al. The multifarious hippocampal mossy fiber pathway: a review. *Neuroscience.* 2000; 98:407–427. [PubMed: 10869836]
46. Nicoll RA, Schmitz D. Synaptic plasticity at hippocampal mossy fibre synapses. *Nat. Rev. Neurosci.* 2005; 6:863–876. [PubMed: 16261180]
47. Rollenhagen A, Lubke JH. The mossy fiber bouton: the "common" or the "unique" synapse. *Front Synaptic Neurosci.* 2010; 2:2. [PubMed: 21423488]
48. Chicurel ME, Harris KM. Three-dimensional analysis of the structure and composition of CA3 branched dendritic spines and their synaptic relationships with mossy fiber boutons in the rat hippocampus. *J. Comp. Neurol.* 1992; 325:169–182. [PubMed: 1460112]
49. Acsady L, et al. GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *J. Neurosci.* 1998; 18:3386–3403. [PubMed: 9547246]
50. Rollenhagen A, et al. Structural determinants of transmission at large hippocampal mossy fiber synapses. *J. Neurosci.* 2007; 27:10434–10444. [PubMed: 17898215]
51. Hallermann S, et al. A large pool of releasable vesicles in a cortical glutamatergic synapse. *Proc. Natl. Acad. Sci. U. S. A.* 2003; 100:8975–8980. [PubMed: 12815098]
52. Lawrence JJ, et al. Quantal transmission at mossy fibre targets in the CA3 region of the rat hippocampus. *J. Physiol.* 2004; 554:175–193. [PubMed: 14678500]
53. Li L, et al. Differential gating and recruitment of P/Q-, N-, and R-type Ca^{2+} channels in hippocampal mossy fiber boutons. *J. Neurosci.* 2007; 27:13420–13429. [PubMed: 18057200]
54. Bischofberger J, et al. Timing and efficacy of Ca^{2+} channel activation in hippocampal mossy fiber boutons. *J. Neurosci.* 2002; 22:10593–10602. [PubMed: 12486151]
55. Kempermann G, et al. Experience-induced neurogenesis in the senescent dentate gyrus. *J. Neurosci.* 1998; 18:3206–3212. [PubMed: 9547229]
56. von Bohlen und Halbach O. Immunohistological markers for staging neurogenesis in adult hippocampus. *Cell Tissue Res.* 2007; 329:409–420. [PubMed: 17541643]

57. von Bohlen und Halbach O. Immunohistological markers for proliferative events, gliogenesis, and neurogenesis within the adult hippocampus. *Cell Tissue Res.* 2011; 345:1–19. [PubMed: 21647561]
58. Faulkner RL, et al. Development of hippocampal mossy fiber synaptic outputs by new neurons in the adult brain. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:14157–14162. [PubMed: 18780780]
59. Schinder AF, Gage FH. A hypothesis about the role of adult neurogenesis in hippocampal function. *Physiology (Bethesda).* 2004; 19:253–261. [PubMed: 15381753]
60. Marin-Burgin A, et al. Unique processing during a period of high excitation/inhibition balance in adult-born neurons. *Science.* 2012; 335:1238–1242. [PubMed: 22282476]
61. Zador A. Impact of synaptic unreliability on the information transmitted by spiking neurons. *J. Neurophysiol.* 1998; 79:1219–1229. [PubMed: 9497403]
62. Hardingham NR, Larkman AU. Rapid report: the reliability of excitatory synaptic transmission in slices of rat visual cortex in vitro is temperature dependent. *J. Physiol.* 1998; 507:249–256. [PubMed: 9490846]
63. Goda Y, Sudhof TC. Calcium regulation of neurotransmitter release: reliably unreliable. *Curr. Opin. Cell Biol.* 1997; 9:513–518. [PubMed: 9261057]
64. Zhai RG, et al. Assembling the presynaptic active zone: a characterization of an active one precursor vesicle. *Neuron.* 2001; 29:131–143. [PubMed: 11182086]
65. Sorra KE, et al. Dense core vesicles resemble active-zone transport vesicles and are diminished following synaptogenesis in mature hippocampal slices. *Neuroscience.* 2006; 141:2097–2106. [PubMed: 16797135]
66. Yazejian B, et al. Direct measurements of presynaptic calcium and calcium-activated potassium currents regulating neurotransmitter release at cultured *Xenopus* nerve-muscle synapses. *J. Neurosci.* 1997; 17:2990–3001. [PubMed: 9096135]
67. Yazejian B, et al. Tracking presynaptic Ca^{2+} dynamics during neurotransmitter release with Ca^{2+} -activated K^{+} channels. *Nat. Neurosci.* 2000; 3:566–571. [PubMed: 10816312]
68. Han Y, et al. RIM determines Ca^{2+} channel density and vesicle docking at the presynaptic active zone. *Neuron.* 2011; 69:304–316. [PubMed: 21262468]
69. Kaeser PS, et al. RIM proteins tether Ca^{2+} channels to presynaptic active zones via a direct PDZ-domain interaction. *Cell.* 2011; 144:282–295. [PubMed: 21241895]
70. Bezprozvanny I, et al. Functional impact of syntaxin on gating of N-type and Q-type calcium channels. *Nature.* 1995; 378:623–626. [PubMed: 8524397]
71. Atlas D. Functional and physical coupling of voltage-sensitive calcium channels with exocytotic proteins: ramifications for the secretion mechanism. *J. Neurochem.* 2001; 77:972–985. [PubMed: 11359862]
72. Yokoyama CT, et al. Mechanism of SNARE protein binding and regulation of Cav2 channels by phosphorylation of the synaptic protein interaction site. *Mol. Cell. Neurosci.* 2005; 28:1–17.
73. Keith RK, et al. Bidirectional modulation of transmitter release by calcium channel/syntaxin interactions in vivo. *J. Neurosci.* 2007; 27:265–269. [PubMed: 17215385]
74. Pozzi D, et al. Activity-dependent phosphorylation of Ser187 is required for SNAP-25-negative modulation of neuronal voltage-gated calcium channels. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:323–328. [PubMed: 18162553]
75. Yang YM, et al. Septins regulate developmental switching from microdomain to nanodomain coupling of Ca^{2+} influx to neurotransmitter release at a central synapse. *Neuron.* 2010; 67:100–115. [PubMed: 20624595]
76. Feldmeyer D, Radnikow G. Developmental alterations in the functional properties of excitatory neocortical synapses. *J. Physiol.* 2009; 587:1889–1896. [PubMed: 19273572]
77. Golshani P, et al. Internally mediated developmental desynchronization of neocortical network activity. *J. Neurosci.* 2009; 29:10890–10899. [PubMed: 19726647]
78. Rochefort NL, et al. Sparsification of neuronal activity in the visual cortex at eye-opening. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106:15049–15054. [PubMed: 19706480]
79. Crins TT, et al. Developmental changes in short-term plasticity at the rat calyx of Held synapse. *J. Neurosci.* 2011; 31:11706–11717. [PubMed: 21832200]

80. Eggermann E, et al. Nanodomain coupling between Ca(2) channels and sensors of exocytosis at fast mammalian synapses. *Nat. Rev. Neurosci.* 2012; 13:7–21. [PubMed: 22183436]
81. Simon SM, Llinas RR. Compartmentalization of the submembrane calcium activity during calcium influx and its significance in transmitter release. *Biophys. J.* 1985; 48:485–498. [PubMed: 2412607]
82. Llinas R, et al. Microdomains of high calcium concentration in a presynaptic terminal. *Science.* 1992; 256:677–679. [PubMed: 1350109]
83. Llinas R, et al. Transmission by presynaptic spike-like depolarization in the squid giant synapse. *Proc. Natl. Acad. Sci. U. S. A.* 1982; 79:2415–2419. [PubMed: 6954549]
84. Neher E, Sakaba T. Multiple roles of calcium ions in the regulation of neurotransmitter release. *Neuron.* 2008; 59:861–872. [PubMed: 18817727]
85. Dodge FA Jr, Rahamimoff R. Co-operative action a calcium ions in transmitter release at the neuromuscular junction. *J. Physiol.* 1967; 193:419–432. [PubMed: 6065887]
86. Gaffield MA, et al. The spatial pattern of exocytosis and post-exocytic mobility of synaptopHluorin in mouse motor nerve terminals. *J. Physiol.* 2009; 587:1187–1200. [PubMed: 19153160]
87. Koester HJ, Sakmann B. Calcium dynamics associated with action potentials in single nerve terminals of pyramidal cells in layer 2/3 of the young rat neocortex. *J. Physiol.* 2000; 529:625–646. [PubMed: 11118494]
88. Brandt A, et al. Few CaV1.3 channels regulate the exocytosis of a synaptic vesicle at the hair cell ribbon synapse. *J. Neurosci.* 2005; 25:11577–11585. [PubMed: 16354915]
89. Goutman JD, Glowatzki E. Time course and calcium dependence of transmitter release at a single ribbon synapse. *Proc. Natl. Acad. Sci. U. S. A.* 2007; 104:16341–16346. [PubMed: 17911259]
90. Jarsky T, et al. Nanodomain control of exocytosis is responsible for the signaling capability of a retinal ribbon synapse. *J. Neurosci.* 2010; 30:11885–11895. [PubMed: 20826653]
91. Bartoletti TM, et al. Release from the cone ribbon synapse under bright light conditions can be controlled by the opening of only a few Ca(2+) channels. *J. Neurophysiol.* 2011; 106:2922–2935. [PubMed: 21880934]
92. Raingo J, et al. VAMP4 directs synaptic vesicles to a pool that selectively maintains asynchronous neurotransmission. *Nat Neurosci.* 2012; 15:738–745. [PubMed: 22406549]
93. Mutch SA, et al. Protein quantification at the single vesicle level reveals that a subset of synaptic vesicle proteins are trafficked with high precision. *J. Neurosci.* 2011; 31:1461–1470. [PubMed: 21273430]
94. Frank T, et al. Mechanisms contributing to synaptic Ca²⁺ signals and their heterogeneity in hair cells. *Proc. Natl. Acad. Sci. U S A.* 2009; 106:4483–4488. [PubMed: 19246382]

Box 1: Nanodomain vs. Microdomain coupling of Ca^{2+} to transmitter release

To aid in the discussion of sub-active zone mechanisms for transmitter release, we compare and contrast two concepts that are often used when discussing Ca^{2+} -triggered vesicle fusion: nanodomain and microdomain coupling of Ca^{2+} to transmitter release (Figure I) [80]. These concepts are used to classify Ca^{2+} signals which synaptic vesicles are exposed to during an AP into two main categories. Nanodomain coupling refers to vesicle release triggered by the localized Ca^{2+} signal in close vicinity to the mouth of a single Ca^{2+} channel after activation (Figure Ia). Cellular imaging and computational modeling of single Ca^{2+} channels predict that the Ca^{2+} concentration in such a nanodomain can be as high as hundreds of μM [81,82]. However, since the spatial extent of the nanodomain is so small, the actual number of Ca^{2+} ions present is very small, typically only a few hundred ions [10]. During nanodomain-coupled vesicle release, a synaptic vesicle is located within this Ca^{2+} nanodomain and the Ca^{2+} concentration is sufficiently high to trigger vesicle fusion and transmitter release [10, 83]. Under this scenario, only a fast-binding Ca^{2+} buffer, like BAPTA, can effectively compete with the Ca^{2+} sensor for transmitter release.

On the other hand, microdomain Ca^{2+} signals are produced by the summed, overlapping Ca^{2+} clouds generated by Ca^{2+} entry through multiple open Ca^{2+} channels that are not tightly associated with one another or the vesicle release sites in an active zone (essentially increasing background Ca^{2+} near the AZ; Figure Ib), with a spatial extent ranging from 100 nm to 1 μm [84]. When transmitter release is triggered by a microdomain of Ca^{2+} , many channels need to open to generate a Ca^{2+} concentration near vesicles that is high enough to cause vesicle fusion. In this scenario, even relatively slow binding buffers like EGTA (in addition to BAPTA), can effectively compete with the Ca^{2+} sensor for transmitter release.

Nanodomain and microdomain Ca^{2+} signals may be mixed at some synapses due to a range of Ca^{2+} channel to vesicle release site distances. This type of non-uniform topographic relationship would produce a large variability in the Ca^{2+} transient sensed by different vesicles, resulting in heterogeneity of their release probability during an AP. Indeed, a numerical model that reproduced most experimental data of the immature calyx of Held synapse predicted that vesicles located at various distances would be released with probabilities that are inversely related to coupling distances, and ranged from 100% to less than 1% [38].

Box 2: Ca²⁺ channel - release site relationship

The “Ca²⁺ channel-release site relationship” is often used to help distinguish between nanodomain and microdomain coupling of Ca²⁺ flux to transmitter release. The approach is based on the argument that if the fusion of each synaptic vesicle is triggered by the Ca²⁺ flux through a single channel, then as the number of these channels is varied experimentally (e.g. by blocking with toxins or changing the shape of the voltage depolarization used to activate channels), the change in magnitude of transmitter release is expected to be linear (despite the known fourth order relationship between extracellular Ca²⁺ concentration and transmitter release; also called molecular cooperativity; [85]). On the other hand, if the fusion of each synaptic vesicle is triggered by the Ca²⁺ flux through many open Ca²⁺ channels, then as the number of these channels activated is varied, the relationship between transmitter release magnitude and the number of open Ca²⁺ channels will be non-linear (with large changes in transmitter release after relatively small changes in Ca²⁺ entry), and is often similar to the molecular cooperativity (4th order). This occurs because reducing the number of channels at single vesicle release sites that contain many channels has an effect on the overall Ca²⁺ flux that is similar to reducing extracellular Ca²⁺.

Box 3. Outstanding Questions

- What mechanisms govern the low probability of release from unreliable single vesicle release sites

In part, we hypothesize this to be governed by the low probability that Ca^{2+} channels open near these release sites. While at the frog NMJ there may only be a single Ca^{2+} channel positioned close to each single vesicle release site, the precise stoichiometric relationship between Ca^{2+} channels and vesicle release sites at this and other synapses requires further study; it is certainly possible that there is heterogeneity in this relationship. Further, even though Ca^{2+} channels can be very close to synaptic vesicles, we hypothesize a low probability that vesicles will fuse following exposure to Ca^{2+} flux from a nearby Ca^{2+} channel. An elucidation of mechanisms controlling the probability of release from single vesicle release sites requires additional experimental work.

- Is there significant heterogeneity in release probability, and the underlying sub-active zone mechanisms that control transmitter release, across different active zones at large multi-active zone synapses

At mouse NMJs, there is evidence for significant heterogeneity in release probability across different AZs within an individual nerve terminal [86]. Therefore, this issue is likely to be an important one and will require further study at many synapses.

- How common are unreliable single vesicle release sites in the CNS

An important set of synapses that requires further investigation is the large heterogeneous population of small cortical bouton-like synapses. Previous studies have shown that transmitter release from these small CNS synapses is often unreliable [63]. In studies that have been performed to date, significant variability exists when measuring the average Ca^{2+} influx in response to a single AP among different boutons of a single presynaptic neuron [87]. However, when studying the variability in Ca^{2+} influx at a single bouton, some have reported a high degree of variability (consistent with a low probability of Ca^{2+} channel opening following AP stimuli; [20]), while others have observed a homogeneous Ca^{2+} influx with repeated stimulus trials (consistent with either a large number of Ca^{2+} channels and/or a high probability of Ca^{2+} channel opening following AP stimuli; [87]). Additional studies will be required to better understand Ca^{2+} channel number and function at this diverse set of synapses.

- How are single vesicle release sites controlled at synapses that do not use APs to trigger calcium influx and transmitter release

Ribbon synapses are unique synapses that release large amounts of transmitter over extended periods of time in response to graded potentials. A number of studies suggest that single vesicle release sites at ribbon synapses employ nanodomain control of vesicle fusion [88–91].

- As a final point, while we have focused our discussion on the ways in which different types of synapses may be assembled by varying the number and spatial organization of unreliable single vesicle release sites, many other factors undoubtedly contribute significantly

The roles of synaptic vesicle pools, as well as potential molecular heterogeneity in synaptic vesicle protein constituents, active zone components, and presynaptic calcium channel subunits [92–94], require additional study.

Figure 1.

Hypothetical model based on the unreliable single vesicle release site as a building block of mature synapses. Synaptic elements (Ca^{2+} channels and synaptic vesicles) are depicted at the time of AP invasion of the nerve terminal. (a) Recent data has led to a model for synaptic assembly based on unreliable single vesicle release sites. These consist of a synaptic vesicle, sparse numbers of closely associated Ca^{2+} channels that each open with a low probability during an AP stimulus, and vesicle fusion that is triggered by the nanodomain of Ca^{2+} flux from as few as one tightly associated Ca^{2+} channel (dark blue

regions near the mouth of an open channel). Low release probability synapses can be constructed using a small number of these basic building blocks within few active zones. On the other hand, large and reliable synapses can be constructed using large numbers of the same unreliable single vesicle release site within hundreds of active zones. Variation in the number and size of active zones in these larger synapses, and the number and spatial organization of the unreliable single vesicle release sites within the active zones, likely contributes to differences in synaptic properties. The top half of this figure shows representative examples of synapses that appear to be constructed in this manner (ie. dentate gyrus basket cell boutons, mature calyx of Held synapses, frog NMJ, and mouse NMJ). **(b)** In contrast, some CNS synapses appear to be assembled with a large number of presynaptic Ca^{2+} channels that are loosely coupled to synaptic vesicles and open with a relatively high probability in response to an AP stimulus. Vesicle fusion at these synapses is triggered by the microdomain of Ca^{2+} that is created by the flux through many loosely coupled Ca^{2+} channels, essentially increasing background Ca^{2+} in the active zone region (summed light blue regions). The bottom half of this figure shows representative examples of synapses that appear to be constructed in this manner (ie. boutons of cerebellar parallel fibers and immature calyx of Held synapses). Evidence also suggests that during development some synapses initially assembled using microdomain-coupled vesicle release sites may mature into synapses that use unreliable single vesicle release sites [33, 36, 39].

(a) Nanodomain coupling

(b) Microdomain coupling

SV = synaptic vesicle; closed Ca^{2+} channel = ; open Ca^{2+} channel =
 nanodomain near an open Ca^{2+} channel = ; elevated background calcium =

Box 1 Figure I.

Schematic diagram that compares microdomain and nanodomain coupling of a single vesicle release site to Ca^{2+} channels. **(a)** Nanodomain coupling occurs when vesicle fusion at single vesicle release sites is triggered by the local Ca^{2+} flux through a single or very small number of open Ca^{2+} channel(s) (dark blue regions). **(b)** Microdomain coupling occurs when vesicle fusion at single vesicle release sites is triggered by the summed Ca^{2+} flux through many loosely coupled Ca^{2+} channels that essentially increases background Ca^{2+} in the active zone region (summed light blue regions).