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The ubiquitous nature of multivesicular release

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Abstract

“Simplicity is prerequisite for reliability.” EW Dijkstra [1]

Presynaptic action potentials trigger the fusion of vesicles to release neurotransmitter onto postsynaptic neurons. Each release site was originally thought to liberate at most one vesicle per action potential in a probabilistic fashion, rendering synaptic transmission unreliable. However, the simultaneous release of several vesicles, or multivesicular release (MVR), represents a simple mechanism to overcome the intrinsic unreliability of synaptic transmission. MVR was initially identified at specialized synapses but is now known to be common throughout the brain. MVR determines the temporal and spatial dispersion of transmitter, controls the extent of receptor activation, and contributes to adapting synaptic strength during plasticity and neuromodulation. MVR consequently represents a widespread mechanism that extends the dynamic range of synaptic processing.

Multivesicular release occurs throughout the brain

Fast chemical communication between neurons occurs at ultrastructurally-defined synaptic junctions through the release of neurotransmitters. At each presynaptic release site, neurotransmitter-filled vesicles are docked on the plasma membrane ready to fuse upon the arrival of an action potential (Figure 1Ai and Bi). Vesicle fusion and neurotransmitter release then results in receptor activation. The strength of the synaptic signal at the postsynaptic membrane is determined by the number of release sites (N) see Glossary, the probability that a vesicle is released (P_r), and the amplitude of the postsynaptic response elicited by the content of each synaptic vesicle (q) [2]. Seminal work correlating morphology with physiology led to the idea that an action potential allows the stochastic fusion of, *at most*, one vesicle at each release site (univesicular release; UVR) [3–8]. Under this model, the maximum number of vesicles released corresponds to the number of

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anatomically defined release sites, N [9]. The tenet of UVR was thought to apply to most synapses (for review see [10]); however, several observations are inconsistent with the ‘one site, one vesicle’ hypothesis, including considerable amplitude fluctuations of evoked synaptic responses [11] and high transmitter concentration in the synaptic cleft [12]. To reconcile these findings, a more flexible hypothesis proposes that multiple vesicles can be released per active zone with each action potential, defined as multivesicular release (MVR). Indeed, studies have established that MVR occurs at many inhibitory and excitatory synapses throughout the brain, including hippocampus, cerebral cortex, cerebellum, hypothalamus, and at sensory synapses [12–21]. Due to the sub-millisecond speed of vesicle fusion direct electron microscopic evidence of multiple vesicle fusions (omega figures) at single release sites has been rare (but see [22, 23]; Figure 1). Nevertheless, functional data from many studies strongly suggests that MVR is a widespread phenomenon among synapses - more prevalent than originally assumed.

In this review, we delineate the evidence and consequences of MVR, including its effects on the time course and concentration of transmitter, receptor occupancy and desensitization. We will examine the experimental approaches that have helped identify MVR, explore how MVR shapes synapse properties, and argue that MVR not only promotes synaptic strength and reliability, but also enhances a neuron’s dynamic range during plasticity and neuromodulation [24, 25]. Finally, we will review the role of MVR in a wide range of physiological contexts, underlining MVR as an indispensable mechanism for neuronal computation.

How MVR Shapes Synaptic Transmission

Receptor Occupancy and Transmitter Concentration

Heterogeneity in synapse size, vesicular transmitter content, transmitter clearance, and receptor affinity contribute to the variability of neurotransmitter-receptor occupancy levels [26, 12, 27–30]. MVR can only increase strength at individual synapses when receptor occupancy by transmitter released from a single vesicle is sufficiently low. Indeed, evidence from many synapses suggests that transmitter from one vesicle is unlikely to fully occupy postsynaptic receptors [31, 32, 11]. For example, at excitatory hippocampal synapses, transmitter from multiple vesicles can interact with a common population of postsynaptic receptors to increase synaptic strength (Figure 1B), and P_r determines the extent of receptor occupancy [12]. These observations are consistent with several studies showing increased receptor occupancy under high- P_r conditions [33–35]. Low receptor occupancy is particularly evident between cholecystokinin (CCK)-positive interneurons and CA3 pyramidal cells where MVR enhances the inhibitory postsynaptic current (IPSC) by several fold [30]. Hence, MVR allows synapses with low receptor occupancy to enhance their dynamic range. However, the extent of receptor occupancy varies markedly across synapses. For example, the effect of MVR on IPSC amplitude is minimal at synapses between cerebellar molecular interneurons (MLIs), implying near full occupancy of postsynaptic receptors by the contents of a single vesicle [13]. While such high-occupancy synapses forfeit dynamic range due to insensitivity to changes in P_r , they operate with greater fidelity.

Together, these examples demonstrate that the degree of receptor occupancy is an important consideration in assessing the consequences of MVR.

The Temporal and Spatial Spread of Transmitter

Evoked transmission across synapses is typically tightly timed to the presynaptic stimulation [36] suggesting temporal coordination across release sites (Figure 1C, left). Yet, MVR has been reported to be either highly coordinated [37, 16] (Figure 1C, center), or desynchronous [38, 39] in a manner that alters the neurotransmitter concentration at postsynaptic receptors (Figure 1C). Numerical simulations suggest that desynchronization of MVR can differentially influence glutamatergic α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and N-methyl-D-aspartate receptor (NMDAR) activation [40]. Near-synchronous MVR (tens of μ sec) increases AMPAR activation supra-linearly, while more pronounced temporal dispersion of MVR (several msec) can result in AMPAR desensitization. Conversely, synchrony has little effect on NMDAR activation. Therefore, activity-dependent desynchronization of MVR might not only alter the time course of the synaptic currents but also its relative receptor contributions. Although the molecular mechanisms underlying MVR are currently unknown, it is tempting to speculate how it regulates both synaptic integration and recruitment of downstream signaling.

The elevated transmitter concentrations that result from MVR can, under some circumstances when transmitter clearance is impeded by restricted geometry, promote rebinding of transmitter to synaptic receptors [41]. MVR can also promote transmitter escape from the synaptic cleft to activate extrasynaptic receptors [42–46, 17]. Both rebinding and spillover are expected to prolong the duration of synaptic currents, and indeed there is a correlation between MVR and increased decay time [47, 30]. Transmitter spillover most likely occurs when the transmitter concentration is high, e.g. after MVR, when release sites are closely spaced, or in the absence of glial diffusion barriers and uptake machinery [48–51]. A clear distinction between spillover from a neighboring release site and MVR is often difficult because both phenomena can result in prolonged presence of transmitter in the synaptic cleft and at extrasynaptic sites [52–54]. However, studies that use low-affinity antagonists suggest that spillover does not increase peak transmitter concentration in the cleft while MVR does [14, 17], allowing pharmacological distinction when release sites are sufficiently far apart. At many synapses, glial cell processes that enwrap synapses establish an anatomical diffusion barrier and provide high densities of excitatory amino acid transporters (EAATs) to separate release sites. Together with perisynaptic neuronal EAATs, these transporters rapidly buffer and uptake glutamate to spatially and temporally confine transmitter spread [55] and maintain extracellular glutamate concentrations in the nM range [56].

In accord with experimental findings that only high levels of MVR can temporarily overwhelm glutamate clearance mechanisms [57, 46], and that spillover is more apparent at synapses lacking glial ensheathment [58], simulations suggest that synapse-crosstalk occurs only when nearsynchronous MVR is combined with a lack of astrocytic coverage [40, 59]. Hence, although MVR significantly increases transmitter concentration, efficient clearance largely eliminates spillover, highlighting that independence of glutamatergic synapses

remains preserved even during MVR. As a result, at excitatory synapses with low MVR such as SC-CA1 contacts, spillover is most evident in response to repetitive stimulation and when monitored with high-affinity, slowly desensitizing NMDARs that are more suitable to detect lower glutamate concentrations [60, 44]. However, the nearsimultaneous stimulation of adjacent fibers that occurs with many experimental paradigms may exaggerate the consequences of spillover [44, 61]. A notable exception is glutamate spillover from the climbing fiber (CF) to MLIs in the cerebellar cortex. Here, high MVR allows glutamate to activate interneuron AMPARs and NMDARs, generating slow and long-lasting excitation [62] that increases firing for tens of milliseconds (see below and [63, 64]).

Another potential consequence of prolonged transmitter presence following MVR is receptor desensitization. Desensitization can diminish receptor availability, contribute to short-term depression, shape the time course of synaptic currents, and is most common among synapses with clustered release sites, high P_r and slowed transmitter clearance [65–68]. Hence, desensitization occurs at some but not all MVR synapses. For example, there is minimal desensitization of AMPARs at synapses where glutamate is rapidly cleared by glial and neuronal EAATs (CF $\rightarrow$ Purkinje cell (CF $\rightarrow$ PC) synapses) [69, 70], whereas MVR results in profound desensitization at retinogeniculate contacts [51]. In conclusion, MVR can exacerbate desensitization, but synapse tortuosity, glial coverage, receptor composition and spacing of release sites are likely the main factors [71, 72]. Less is known about how MVR of gamma-aminobutyric acid (GABA) affects spillover and desensitization. However, the lack of efficient clearance mechanisms predicts a greater extent of GABA escape from the synaptic cleft, perhaps with more pronounced effects on GABA_A receptor desensitization and tonic inhibitory currents [73–75].

Vesicle Release Probability as Predictor of MVR?

What is the principal determinant of MVR? The most parsimonious hypothesis is that P_r regulates whether multiple vesicles are released concurrently. If each docked vesicle can fuse independently in response to an action potential, then the simultaneous release of multiple vesicles will occur more readily at synapses with higher P_r . For example, if two docked vesicles are each released with a probability of 0.9, then the simultaneous release of two vesicles will occur with a probability of 0.81. Consistent with this idea, the exceptionally high P_r at the CF terminal (~ 0.35 – 0.9) [76, 77] coincides with significant MVR. Yet, some high- P_r synapses display exclusively UVR [6, 7], whereas various low- P_r synapses exhibit MVR [15, 34, 17, 78] demonstrating that P_r does not always reliably predict the incidence of MVR.

However, increasing P_r promotes MVR—Many low- P_r synapses generate small UVR postsynaptic currents (PSCs) prone to failure, yet repetitive stimulation can facilitate PSC amplitude. Release models predict that this facilitation depends on an increase in the number of fusion-ready vesicles and/or P_r [79]. Activity-dependent long and short-term increases of MVR, e.g. during facilitation, augmentation and long-term potentiation (LTP), have been observed at numerous synapses with low initial P_r [11, 34, 17, 80, 81], supporting the view that high P_r promotes MVR. Considering inter-terminal heterogeneity in synapse size, P_r , number of docked vesicles and molecular make-up [82–84], it is also probable that the

extent of MVR differs strongly from terminal to terminal [85, 78]. It is therefore important to note that although most central synapses have low P_r , mechanisms that elevate P_r , such as frequency facilitation, decreased presynaptic inhibition, or presynaptically expressed LTP, may increase the probability of MVR [14, 15, 11, 34, 17, 80, 81].

Functions of MVR throughout the brain

High-fidelity transmission and adaption at sensory synapses: MVR par excellence at synaptic ribbons

Sensory processing requires neuronal circuits to transform graded information into a temporal code of action potentials. To continuously perform these computations synapses need to adapt to sustained levels of transmitter release. Unlike conventional synapses that require action potentials to release vesicles, the presynaptic cells at many primary sensory synapses typically operate via graded membrane potential changes that can last several seconds. Here, vesicles are often tethered to a specialized protein structure called a ribbon that is thought to maintain and synchronize the release of hundreds of vesicles per second [86–89, 16 but see, 90]. For example, at retinal bipolar cell terminals a 200 ms depolarization can release ~6000 synaptic vesicles at 50 synaptic ribbon release sites, with each ribbon releasing ~120 vesicles, a number similar to the total number of docked vesicles at the ribbon plus those tethered to the ribbon [91]. In fact, vesicle pool depletion is thought to be the major mechanism for spike adaption during sensory stimuli [92, 93]. Release can occur with rapid and slow kinetics at ribbon synapses, resulting in multiphasic and/or large-amplitude excitatory postsynaptic currents (EPSCs) [94, 89, 95]. Several hypotheses have been proposed to explain the nearsynchronous fusion of several tens of vesicles, including coordination of release sites and tight coupling of calcium channels to the release machinery [89, 96, 21], fusion of several vesicles to each other prior to exocytosis (compound fusion [97]). Recently, however, fusion pore regulation of UVR events has been proposed as an alternate mechanism to explain multiphasic EPSCs [90].

MVR is prominent throughout the retinal circuit. Rod bipolar cells (RBCs) receive input from light-sensing rods and relay the visual information to AII amacrine cells. This retinal ribbon synapse exhibits highly synchronous MVR [16] that is modulated by the number of open calcium channels. MVR requires at least two open calcium channels to trigger the release of multiple vesicles from one ribbon release site [98]. Astoundingly, transmitter from the 2–4 simultaneously released vesicles onto amacrine cells neither fully occupies nor desensitizes postsynaptic AMPARs, allowing for linear summation of each vesicle and a wide range of synaptic operation [93, 99]. In contrast, MVR at cone bipolar cell-ganglion cell synapses fully occupies AMPARs and activates perisynaptically located NMDARs in a concentration-dependent manner [45, 100, 101]. In addition, at some bipolar cell terminals presynaptic activation of GABA_C receptors regulates P_r and MVR and determines the extent of spillover and NMDAR activation [102, 103, 100].

In summary, sensory synapses take advantage of MVR to regulate firing duration and frequency, and to ensure reliable and sustained postsynaptic firing at high frequencies. Moreover, MVR at ribbon synapses results in the release of thousands of vesicles at single active zones over prolonged periods of stimulation. The synaptic ribbon therefore appears to

be a highly specialized structure that promotes continuous MVR at primary sensory synapses.

MVR at excitatory and inhibitory hippocampal synapses

Although initial studies rejected the MVR hypothesis [104, 105], considerable variability in postsynaptic currents, anatomical and molecular properties led to the speculation that hippocampal synapses use MVR to increase their dynamic range [106, 83, 11, 30] (see Box 1 and Figure 2). Indeed, modeling of SC-CA1 excitatory PSCs (EPSCs) also favors the idea that MVR underlies current amplitude fluctuations [29, 107]. A pioneering study in cultured hippocampal neurons identified MVR based on observations that multiple quanta interact with a common population of postsynaptic receptors [12]. Consistent with this notion, NMDAR-mediated calcium influx at single SC-CA1 spines increases in a step-wise manner during repetitive stimulation, demonstrating MVR underlies enhanced synaptic potency during facilitation [15, 11, 38]. Additionally, evidence from electrophysiological studies suggests that cleft glutamate concentration increases during trains of stimuli [17]. Finally, presynaptic modulators can control MVR in an input- and state-specific manner. For example, activation of the presynaptic estrogen receptor beta (ER β) induces acute potentiation of MVR specifically at low P_r SC-CA1 contacts [108]. Moreover, the transition from UVR to MVR may underlie the expression of late LTP at CA3-CA1 synapses [109]. Together these studies converge on the view that MVR dynamically promotes reliable transmission at typically low- P_r hippocampal synapses and contributes to activity- and context-dependent plasticity.

Increasing Reliability of Cortical and Subcortical Synapses

Heterogeneity of release mechanisms prevails in the cerebral cortex. Studies using statistical arguments and anatomical reconstructions find evidence for UVR at intracortical excitatory synapses [110, 111, 6]. But MVR can also enable faithful transmission at intracortical synapses in layer 4 of the somatosensory cortex. Here, L4 neuron terminals display high P_r and postsynaptic receptor saturation that reliably initiate action potentials, although MVR is less prevalent at L4 connections in the visual cortex [19]. It is possible that the differential expression of MVR represents distinct coding of information depending on content or valence.

Thalamic input to the cortex recruits robust feed-forward inhibition critical for limiting cortical excitation and refining sensory processing [112, 113]. High- P_r thalamocortical synapses onto interneurons display significant MVR (up to 7 vesicles), driving reliable inhibition onto cortical pyramidal neurons [114]. However, the close spacing of release sites hampers a clear distinction between MVR and spillover at these contacts. In addition, corticothalamic projections from layer 6 pyramidal neurons show use-dependent enhancement of synaptic strength mediated by increased P_r , MVR and, consequently, receptor saturation [81]. In conclusion, while intracortical processing seems to rely primarily on UVR synapses, evidence suggests that MVR is important at the input and output stages where signal transmission requires greater reliability.

In the striatum, cholinergic modulation of MVR and low levels of receptor saturation enhance the dynamic range of excitatory inputs onto medium spiny neurons. MVR is prevalent under basal conditions but acetylcholine release from interneurons dramatically decreases synaptic potency (and MVR) by activating presynaptic muscarinic acetylcholine receptors (mAChRs) [18]. The decrease in MVR accelerates the time course of excitatory postsynaptic potentials (EPSPs), an effect that can be attributed to MVR recruiting local voltage-gated conductances or NMDARs and shows that MVR can control dendritic nonlinearities. Similar to mAChRs, presynaptic GABA_B receptors at cortical pyramidal neurons reduce synaptic potency by decreasing P_r and MVR [115]. Therefore, the modulation of the number of vesicles released at single synaptic sites appears to control local signal integration, in addition to synaptic strength.

Dynamics of Transmitter Release in the Cerebellum

MVR has been studied in detail at cerebellar CF-PC synapses. Following an action potential the near-synchronous fusion of ~3–5 vesicles per release site gives rise to cleft glutamate concentrations of >10 mM that fully occupy AMPARs and generate large EPSCs [14, 33] that result in bursts of action potentials termed complex spikes [116–118]. Characteristic of high P_r , repetitive stimuli deplete synaptic vesicles and cause a decrease in CF-EPSC amplitude [70, 119]. Nonetheless, MVR and saturation are thought to help speed recovery from depression, allowing reliable transmission even during sustained CF activity at physiological frequencies observed *in vivo* (<30% depression at 2 Hz; [120, 121, 39]). Firing at these frequencies also causes a slowing of the EPSC waveform that is consistent with desynchronization of MVR within a release site, termed intra-site asynchrony [39]. Synaptic depression and desynchronization of MVR generate complex spikes that propagate more successfully along the PC axon than during near-synchronous MVR, thereby enhancing information transfer to PC target neurons. Downregulation of MVR by various presynaptic receptors (including GABA_B, adenosine, adrenergic and metabotropic glutamate receptors) [122, 123, 39] can disrupt associative plasticity of parallel fiber (PF) inputs [123], suggesting that modulation of MVR at CF synapses has a role in cerebellar learning.

Despite the lack of direct synaptic connections between CFs and MLIs, recent studies indicate that glutamate spillover from CFs evokes slow AMPAR and NMDAR-mediated EPSCs in MLIs that lead to a prolonged increase in firing rate [62–64]. These findings suggest that MVR can enhance the temporal and spatial spread of CF activation, and provide the mechanistic details for the long-lasting modulation of PC firing rate following CF activation *in vivo* [124, 125].

Desynchronization of MVR on a supra-millisecond time scale is also evident at inhibitory synapses between MLIs [13] where 0 to 3 vesicles can be released per action potential at single release sites [126]. In these electrically compact interneurons, desynchronized IPSCs occurring within time intervals of 5–20 ms could provide long-lasting inhibition that limits the integration of incoming PF-mediated excitation. Consistent with MVR at MLI contacts, MLIs can also release multiple synchronized vesicles onto PCs via a calcium store-dependent mechanism, resulting in miniature IPSCs with exceptionally large amplitude ('maxi-mini-IPSCs') [37].

Finally, at least a subset of low- P_r PF terminals also undergoes MVR during long- and short-term activity-dependent potentiation, albeit with considerable intersynaptic heterogeneity [34, 127, 80, 128, 78]. Although the origin of this heterogeneity is unknown, differences in PF bouton size, calcium dynamics and sensitivity to G-protein-coupled receptor activation have been discussed [129, 130]. The high glutamate concentration generated by MVR is thought to activate extrasynaptic NMDARs on MLIs [78], extrasynaptic metabotropic receptors on PCs and MLIs, as well as activating AMPARs on Bergmann glia [52, 33, 131–133].

MVR during synapse maturation and development

During postnatal development synapses undergo extensive morphological and physiological changes. One prominent and well-studied example is the calyx of Held synapse of the auditory pathway [134]. Morphological refinement (i.e. a transition from a cup-shaped structure to more fenestrated and claw-like morphology) occurs concomitantly with a progressive speeding of the presynaptic action potential that decreases P_r and shortens synaptic delay [135]. MVR is less prominent at the mature compared to the immature calyx [136], allowing for more rapid glutamate clearance and less AMPAR desensitization and saturation [66, 136]. These coordinated changes enable this auditory synapse to fire spikes at high frequencies (up to one KHz). The expression of MVR at a given synapse may therefore be subject to developmental remodeling and track with changes in P_r .

In the developing cerebellum, PCs receive multiple CF inputs that undergo activity-dependent pruning. Strong evidence suggests that the CFs with the greatest extent of MVR evoke the largest depolarization-dependent calcium signals in PC dendrites and consequently evade synapse elimination [137, 138]. The mechanisms that establish MVR prior to pruning remain incompletely understood but these results suggest that MVR may contribute to synapse refinement.

Sensitization of Homeostatic Circuits

Behavioral responses to external stimuli often intensify upon stimulus re-occurrence. Several lines of evidence suggest that MVR may be a synaptic correlate to such behavioral sensitization. One example is the stress response of the hypothalamic-pituitary-adrenal (HPA) axis that generates a long-lasting sensitivity to repetitive stressors [139] and involves enhanced activity of parvocellular neurosecretory cells (PNCs) in the paraventricular nucleus of the hypothalamus. A recent study found potentiated excitatory synaptic transmission onto PNCs in animals pre-exposed to a single stressor compared to naive animals. The mechanism of enhancement is consistent with an activity-dependent depression of NMDAR current that diminishes calcium influx and release of a tonic retrograde inhibitor from PCNs. Disinhibition of transmitter release in turn increases MVR. Remarkably, both behavioral sensitization to the stressor and enhanced MVR last several days [20]. Therefore, potentiation of MVR may be the synaptic correlate of enhanced HPA axis sensitivity.

Noradrenergic modulation of MVR may also regulate hormone secretion from magnocellular neurosecretory cells (MNCs) in the hypothalamus. Noradrenaline augments presynaptic calcium via $\alpha 1$ receptor activation and calcium release from ryanodine-sensitive

stores, transiently enhancing synchronous MVR onto MNCs [140]. In electrically compact cells like MNCs [141] large-amplitude MVR-unitary EPSCs are sufficient to trigger action potential firing and, hence, release of vasopressin and oxytocin, hormones critical in the regulation of hydration, stress, hemorrhage, lactation and parturition. Together, these results demonstrate that neuroendocrine output in the hypothalamus partially underlies the short and long-term regulation of MVR [142].

Concluding Remarks

Here, we present abundant evidence that MVR is a common mechanism for enhancing synaptic reliability and overcoming the stochastic variability of synaptic transmission. Indeed, MVR determines synaptic behavior during repetitive and sustained activation, affects the temporal and spatial activation profile of neurotransmitter receptors and voltage-gated conductances, and can therefore control synaptic integration, downstream intracellular signaling and induction of plasticity. The numerous examples of MVR discussed here illustrate that synapses differentially exploit MVR depending on their functional demands. In conclusion, we suggest that regulation of MVR is a fundamental and simple mechanism for increasing the dynamic range of synaptic transmission and challenge the long-held hypothesis that MVR is a rare phenomenon manifested exclusively at high- P_r contacts.

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Glossary

Active zone	Ultrastructurally and functionally specialized area at the presynaptic terminal where readily releasable vesicles fuse (see release site).
Desynchronization	The temporal jitter of vesicle fusion between synaptic release sites (intersite asynchrony) or within a release site (intrasite asynchrony)
CV	Coefficient of variation; standard deviation σ divided by the mean (e.g. σ of current amplitude fluctuations and mean current). CV is a normalized measure of relative variability within a distribution (e.g. numerous trials of an evoked synaptic current)
Depletion	Temporal unavailability of releasable vesicles due to foregoing release. Vesicular depletion can cause a decrease in synaptic strength
Desensitization	Receptors bound by transmitter but in a non-conducting state can contribute to failure of receptor opening following repeated or prolonged exposure to neurotransmitter. Desensitization can cause a decrease in synaptic strength.

EPSC/EPSP	<u>Excitatory postsynaptic current/potential</u>
IPSC/IPSP	<u>Inhibitory postsynaptic current/potential</u>
Fast-off / Low-affinity antagonist	Rapidly dissociating competitive antagonist
Slow-off / High-affinity antagonist	Slowly dissociating competitive antagonist
MVR	Multivesicular release; the release of >1 vesicles per active zone in response to a single presynaptic action potential. Transmitter released by multiple vesicles interacts with a common population of postsynaptic receptors
P_r	Vesicular release probability; the likelihood that a vesicle fuses upon arrival of an action potential at any release site within an active zone.
Quantal content (<i>m</i>)	All vesicles released in response to an action potential. When quantal content exceeds the number of anatomically defined release sites this is interpreted as MVR
Quantal theory	Postulates that the spontaneously occurring miniature currents/potentials represent the basic element of the evoked synaptic current/potential.
Receptor occupancy	The percentage of receptors bound by released transmitter. 100% occupancy refers to receptor saturation.
Release site	Site within the active zone where a vesicle undergoes fusion. It remains controversial whether release sites are clearly defined molecularly or whether release can take place at any location in the active zone. For the purpose of this review, we define the active zone as a structure that provides release sites, and a single active zone can have multiple release sites.
Spillover	Refers to transmitter escaping the synaptic cleft that activates extrasynaptic receptors located on the releasing cell or receptors on adjacent cells
Synaptic strength	Defines the average response amplitude at a synaptic contact in response to an action potential. The total number of release sites, readily releasable vesicles, P _r and postsynaptic factors, such as single receptor conductance and receptor density, determine synaptic strength
UVR	Univesicular release; the release of 1 vesicle per active zone in response to a single presynaptic action potential
VMA	Variance-mean analysis, a statistical method to estimate synaptic quantal parameters N, P _r and q

References

1. Dijkstra, EW. Selected writings on computing : a personal perspective. Springer-Verlag; 1982.
2. Del Castillo J, Katz B. Quantal components of the end-plate potential. *J Physiol (Lond)*. 1954; 124:560–573. [PubMed: 13175199]
3. Redman S, Walmsley B. Amplitude fluctuations in synaptic potentials evoked in cat spinal motoneurons at identified group Ia synapses. *J Physiol*. 1983; 343:135–145. [PubMed: 6644615]
4. Gulyas AI, et al. Hippocampal pyramidal cells excite inhibitory neurons through a single release site. *Nature*. 1993; 366:683–687. [PubMed: 8259211]
5. Lawrence JJ, McBain CJ. Interneuron diversity series: containing the detonation--feedforward inhibition in the CA3 hippocampus. *Trends Neurosci*. 2003; 26:631–640. [PubMed: 14585604]
6. Silver RA, et al. High-probability unquantal transmission at excitatory synapses in barrel cortex. *Science*. 2003; 302:1981–1984. [PubMed: 14671309]
7. Murphy GJ, et al. Sensory neuron signaling to the brain: properties of transmitter release from olfactory nerve terminals. *J Neurosci*. 2004; 24:3023–3030. [PubMed: 15044541]
8. Biró AA, et al. Quantal size is independent of the release probability at hippocampal excitatory synapses. *J Neurosci*. 2005; 25:223–232. [PubMed: 15634785]
9. Korn H, et al. Fluctuating responses at a central synapse: n of binomial fit predicts number of stained presynaptic boutons. *Science*. 1981; 213:898–901. [PubMed: 6266015]
10. Redman S. Quantal analysis of synaptic potentials in neurons of the central nervous system. *Physiol Rev*. 1990; 70:165–198. [PubMed: 2404288]
11. Conti R, Lisman J. The high variance of AMPA receptor- and NMDA receptormediated responses at single hippocampal synapses: evidence for multiquantal release. *Proc Natl Acad Sci USA*. 2003; 100:4885–4890. [PubMed: 12682300]
12. Tong G, Jahr CE. Multivesicular release from excitatory synapses of cultured hippocampal neurons. *Neuron*. 1994; 12:51–59. [PubMed: 7507341]
13. Auger C, et al. Multivesicular release at single functional synaptic sites in cerebellar stellate and basket cells. *J Neurosci*. 1998; 18:4532–4547. [PubMed: 9614230]
14. Wadiche JI, Jahr CE. Multivesicular release at climbing fiber-Purkinje cell synapses. *Neuron*. 2001; 32:301–313. [PubMed: 11683999]
15. Oertner TG, et al. Facilitation at single synapses probed with optical quantal analysis. *Nat Neurosci*. 2002; 5:657–664. [PubMed: 12055631]
16. Singer JH, et al. Coordinated multivesicular release at a mammalian ribbon synapse. *Nat Neurosci*. 2004; 7:826–833. [PubMed: 15235608]
17. Christie JM, Jahr CE. Multivesicular release at Schaffer collateral-CA1 hippocampal synapses. *J Neurosci*. 2006; 26:210–216. [PubMed: 16399689]
18. Higley MJ, et al. Cholinergic modulation of multivesicular release regulates striatal synaptic potency and integration. *Nat Neurosci*. 2009
19. Huang CH, et al. Multivesicular release differentiates the reliability of synaptic transmission between the visual cortex and the somatosensory cortex. *J Neurosci*. 2010; 30:11994–12004. [PubMed: 20826663]
20. Kuzmiski JB, et al. Stress-induced priming of glutamate synapses unmasks associative short-term plasticity. *Nat Neurosci*. 2010; 13:1257–1264. [PubMed: 20818385]
21. Graydon CW, et al. Sharp Ca(2)(+) nanodomains beneath the ribbon promote highly synchronous multivesicular release at hair cell synapses. *J Neurosci*. 2011; 31:16637–16650. [PubMed: 22090491]
22. Abenavoli A, et al. Multimodal quantal release at individual hippocampal synapses: evidence for no lateral inhibition. *J Neurosci*. 2002; 22:6336–6346. [PubMed: 12151511]
23. Watanabe S, et al. Ultrafast endocytosis at mouse hippocampal synapses. *Nature*. 2013; 504:242–247. [PubMed: 24305055]
24. Tsodyks MV, Markram H. The neural code between neocortical pyramidal neurons depends on neurotransmitter release probability. *Proc Natl Acad Sci U S A*. 1997; 94:719–723. [PubMed: 9012851]

25. Abbott LF, Regehr WG. Synaptic computation. *Nature*. 2004; 431:796–803. [PubMed: 15483601]
26. Bekkers JM, et al. Origin of variability in quantal size in cultured hippocampal neurons and hippocampal slices. *Proc Natl Acad Sci U S A*. 1990; 87:5359–5362. [PubMed: 2371276]
27. Frerking M, et al. Variation in GABA mini amplitude is the consequence of variation in transmitter concentration. *Neuron*. 1995; 15:885–895. [PubMed: 7576637]
28. Ishikawa T, et al. A single packet of transmitter does not saturate postsynaptic glutamate receptors. *Neuron*. 2002; 34:613–621. [PubMed: 12062044]
29. Raghavachari S, Lisman JE. Properties of quantal transmission at CA1 synapses. *J Neurophysiol*. 2004; 92:2456–2467. [PubMed: 15115789]
30. Biró AA, et al. Release probability-dependent scaling of the postsynaptic responses at single hippocampal GABAergic synapses. *J Neurosci*. 2006; 26:12487–12496. [PubMed: 17135411]
31. Mainen ZF, et al. Synaptic calcium transients in single spines indicate that NMDA receptors are not saturated. *Nature*. 1999; 399:151–155. [PubMed: 10335844]
32. McAllister AK, Stevens CF. Nonsaturation of AMPA and NMDA receptors at hippocampal synapses. *Proc Natl Acad Sci U S A*. 2000; 97:6173–6178. [PubMed: 10811899]
33. Harrison J, Jahr CE. Receptor occupancy limits synaptic depression at climbing fiber synapses. *J Neurosci*. 2003; 23:377–383. [PubMed: 12533597]
34. Foster KA, et al. The influence of multivesicular release and postsynaptic receptor saturation on transmission at granule cell to Purkinje cell synapses. *J Neurosci*. 2005; 25:11655–11665. [PubMed: 16354924]
35. Chanda S, Xu-Friedman MA. A low-affinity antagonist reveals saturation and desensitization in mature synapses in the auditory brain stem. *J Neurophysiol*. 2010; 103:1915–1926. [PubMed: 20107122]
36. Katz B, Miledi R. The effect of temperature on the synaptic delay at the neuromuscular junction. *J Physiol (Lond)*. 1965; 181:656–670. [PubMed: 5880384]
37. Llano I, et al. Presynaptic calcium stores underlie large-amplitude miniature IPSCs and spontaneous calcium transients. *Nat Neurosci*. 2000; 3:1256–1265. [PubMed: 11100146]
38. Leitz J, Kavalali ET. Ca(2+)(+) influx slows single synaptic vesicle endocytosis. *J Neurosci*. 2011; 31:16318–16326. [PubMed: 22072683]
39. Rudolph S, et al. Desynchronization of multivesicular release enhances Purkinje cell output. *Neuron*. 2011; 70:991–1004. [PubMed: 21658590]
40. Boucher J, et al. Realistic modelling of receptor activation in hippocampal excitatory synapses: analysis of multivesicular release, release location, temperature and synaptic crosstalk. *Brain structure & function*. 2010; 215:49–65. [PubMed: 20526850]
41. Otis TS, et al. Delayed clearance of transmitter and the role of glutamate transporters at synapses with multiple release sites. *J Neurosci*. 1996; 16:1634–1644. [PubMed: 8774432]
42. Dzubay JA, Jahr CE. The concentration of synaptically released glutamate outside of the climbing fiber-Purkinje cell synaptic cleft. *J Neurosci*. 1999; 19:5265–5274. [PubMed: 10377338]
43. Brasnjo G, Otis TS. Neuronal glutamate transporters control activation of postsynaptic metabotropic glutamate receptors and influence cerebellar long-term depression. *Neuron*. 2001; 31:607–616. [PubMed: 11545719]
44. Arnth-Jensen N, et al. Cooperation between independent hippocampal synapses is controlled by glutamate uptake. *Nat Neurosci*. 2002; 5:325–331. [PubMed: 11896395]
45. Chen S, Diamond JS. Synaptically released glutamate activates extrasynaptic NMDA receptors on cells in the ganglion cell layer of rat retina. *J Neurosci*. 2002; 22:2165–2173. [PubMed: 11896156]
46. Wadiche JI, Jahr CE. Patterned expression of Purkinje cell glutamate transporters controls synaptic plasticity. *Nat Neurosci*. 2005; 8:1329–1334. [PubMed: 16136036]
47. Kondo S, Marty A. Synaptic currents at individual connections among stellate cells in rat cerebellar slices. *J Physiol*. 1998; 509(Pt 1):221–232. [PubMed: 9547395]
48. Scanziani M, et al. Role of intercellular interactions in heterosynaptic long-term depression. *Nature*. 1996; 380:446–450. [PubMed: 8602244]
49. DiGregorio DA, et al. Spillover of glutamate onto synaptic AMPA receptors enhances fast transmission at a cerebellar synapse. *Neuron*. 2002; 35:521–533. [PubMed: 12165473]

50. Takayasu Y, et al. Glial glutamate transporters maintain one-to-one relationship at the climbing fiber-Purkinje cell synapse by preventing glutamate spillover. *J Neurosci.* 2006; 26:6563–6572. [PubMed: 16775144]
51. Budisantoso T, et al. Mechanisms underlying signal filtering at a multisynapse contact. *J Neurosci.* 2012; 32:2357–2376. [PubMed: 22396411]
52. Bergles DE, et al. Glutamate transporter currents in bergmann glial cells follow the time course of extrasynaptic glutamate. *Proc Natl Acad Sci USA.* 1997; 94:14821–14825. [PubMed: 9405697]
53. Bergles DE, Jahr CE. Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron.* 1997; 19:1297–1308. [PubMed: 9427252]
54. Otis TS, et al. Postsynaptic glutamate transport at the climbing fiber-Purkinje cell synapse. *Science.* 1997; 277:1515–1518. [PubMed: 9278516]
55. Tzingounis AV, Wadiche JI. Glutamate transporters: confining runaway excitation by shaping synaptic transmission. *Nat Rev Neurosci.* 2007; 8:935–947. [PubMed: 17987031]
56. Herman MA, Jahr CE. Extracellular glutamate concentration in hippocampal slice. *J Neurosci.* 2007; 27:9736–9741. [PubMed: 17804634]
57. Diamond JS, Jahr CE. Synaptically released glutamate does not overwhelm transporters on hippocampal astrocytes during high-frequency stimulation. *J Neurophysiol.* 2000; 83:2835–2843. [PubMed: 10805681]
58. Carter AG, Regehr WG. Prolonged synaptic currents and glutamate spillover at the parallel fiber to stellate cell synapse. *J Neurosci.* 2000; 20:4423–4434. [PubMed: 10844011]
59. Greget R, et al. Simulation of postsynaptic glutamate receptors reveals critical features of glutamatergic transmission. *PLoS One.* 2011; 6:e28380. [PubMed: 22194830]
60. Asztely F, et al. Extrasynaptic glutamate spillover in the hippocampus: dependence on temperature and the role of active glutamate uptake. *Neuron.* 1997; 18:281–293. [PubMed: 9052798]
61. Marcaggi P, Attwell D. Endocannabinoid signaling depends on the spatial pattern of synapse activation. *Nat Neurosci.* 2005; 8:776–781. [PubMed: 15864304]
62. Szapiro G, Barbour B. Multiple climbing fibers signal to molecular layer interneurons exclusively via glutamate spillover. *Nat Neurosci.* 2007; 10:735–742. [PubMed: 17515900]
63. Mathews PJ, et al. Effects of climbing fiber driven inhibition on Purkinje neuron spiking. *J Neurosci.* 2012; 32:17988–17997. [PubMed: 23238715]
64. Coddington LT, et al. Spillover-mediated feedforward inhibition functionally segregates interneuron activity. *Neuron.* 2013; 78:1050–1062. [PubMed: 23707614]
65. Trussell LO, Fischbach GD. Glutamate receptor desensitization and its role in synaptic transmission. *Neuron.* 1989; 3:209–218. [PubMed: 2576213]
66. Trussell LO, et al. Desensitization of AMPA receptors upon multiquantal neurotransmitter release. *Neuron.* 1993; 10:1185–1196. [PubMed: 7686382]
67. Kinney GA, et al. Prolonged physiological entrapment of glutamate in the synaptic cleft of cerebellar unipolar brush cells. *J Neurophysiol.* 1997; 78:1320–1333. [PubMed: 9310423]
68. Koike-Tani M, et al. Mechanisms underlying developmental speeding in AMPA-EPSC decay time at the calyx of Held. *J Neurosci.* 2005; 25:199–207. [PubMed: 15634782]
69. Dittman JS, Regehr WG. Calcium dependence and recovery kinetics of presynaptic depression at the climbing fiber to Purkinje cell synapse. *J Neurosci.* 1998; 18:6147–6162. [PubMed: 9698309]
70. Hashimoto K, Kano M. Presynaptic origin of paired-pulse depression at climbing fibre-Purkinje cell synapses in the rat cerebellum. *J Physiol (Lond).* 1998; 506:391–405. [PubMed: 9490867]
71. Xu-Friedman MA, Regehr WG. Ultrastructural contributions to desensitization at cerebellar mossy fiber to granule cell synapses. *J Neurosci.* 2003; 23:2182–2192. [PubMed: 12657677]
72. Mugnaini E, et al. The unipolar brush cell: a remarkable neuron finally receiving deserved attention. *Brain Res Rev.* 2011; 66:220–245. [PubMed: 20937306]
73. Rossi DJ, Hamann M. Spillover-mediated transmission at inhibitory synapses promoted by high affinity $\alpha 6$ subunit GABA(A) receptors and glomerular geometry. *Neuron.* 1998; 20:783–795. [PubMed: 9581769]
74. Overstreet, LS., et al. Measuring and modeling the spatiotemporal profile of GABA at the synapse. In: Quick, M., editor. *Transmembrane Transporters.* John Wiley & Sons; 2002. p. 259-275.

75. Bright DP, et al. Profound desensitization by ambient GABA limits activation of delta-containing GABAA receptors during spillover. *J Neurosci.* 2011; 31:753–763. [PubMed: 21228184]
76. Silver RA, et al. Locus of frequency-dependent depression identified with multiple probability fluctuation analysis at rat climbing fibre-Purkinje cell synapses. *J Physiol (Lond).* 1998; 510:881–902. [PubMed: 9660900]
77. Dittman JS, et al. Interplay between facilitation, depression, and residual calcium at three presynaptic terminals. *J Neurosci.* 2000; 20:1374–1385. [PubMed: 10662828]
78. Nahir B, Jahr CE. Activation of extrasynaptic NMDARs at individual parallel fiber-molecular layer interneuron synapses in cerebellum. *J Neurosci.* 2013; 33:16323–16333. [PubMed: 24107963]
79. Zucker RS, Regehr WG. Short-term synaptic plasticity. *Annu Rev Physiol.* 2002; 64:355–405. [PubMed: 11826273]
80. Bender VA, et al. Presynaptically expressed long-term potentiation increases multivesicular release at parallel fiber synapses. *J Neurosci.* 2009; 29:10974–10978. [PubMed: 19726655]
81. Sun YG, Beierlein M. Receptor saturation controls short-term synaptic plasticity at corticothalamic synapses. *J Neurophysiol.* 2011; 105:2319–2329. [PubMed: 21325678]
82. Dobrunz LE, et al. Very short-term plasticity in hippocampal synapses. *Proc Natl Acad Sci U S A.* 1997; 94:14843–14847. [PubMed: 9405701]
83. Schikorski T, Stevens CF. Morphological correlates of functionally defined synaptic vesicle populations. *Nat Neurosci.* 2001; 4:391–395. [PubMed: 11276229]
84. Holderith N, et al. Release probability of hippocampal glutamatergic terminals scales with the size of the active zone. *Nat Neurosci.* 2012; 15:988–997. [PubMed: 22683683]
85. Rosenmund C, et al. Nonuniform probability of glutamate release at a hippocampal synapse. *Science.* 1993; 262:754–757. [PubMed: 7901909]
86. Parsons TD, et al. Calcium-triggered exocytosis and endocytosis in an isolated presynaptic cell: capacitance measurements in saccular hair cells. *Neuron.* 1994; 13:875–883. [PubMed: 7946334]
87. von Gersdorff H, Matthews G. Dynamics of synaptic vesicle fusion and membrane retrieval in synaptic terminals. *Nature.* 1994; 367:735–739. [PubMed: 7906397]
88. Rieke F, Schwartz EA. Asynchronous transmitter release: control of exocytosis and endocytosis at the salamander rod synapse. *J Physiol.* 1996; 493(Pt 1):1–8. [PubMed: 8735690]
89. Glowatzki E, Fuchs PA. Transmitter release at the hair cell ribbon synapse. *Nat Neurosci.* 2002; 5:147–154. [PubMed: 11802170]
90. Chapochnikov NM, et al. Uniquantal release through a dynamic fusion pore is a candidate mechanism of hair cell exocytosis. *Neuron.* 2014; 83:1389–1403. [PubMed: 25199706]
91. von Gersdorff H, et al. Evidence that vesicles on the synaptic ribbon of retinal bipolar neurons can be rapidly released. *Neuron.* 1996; 16:1221–1227. [PubMed: 8663998]
92. Moser T, Beutner D. Kinetics of exocytosis and endocytosis at the cochlear inner hair cell afferent synapse of the mouse. *Proc Natl Acad Sci U S A.* 2000; 97:883–888. [PubMed: 10639174]
93. Singer JH, Diamond JS. Vesicle depletion and synaptic depression at a mammalian ribbon synapse. *J Neurophysiol.* 2006; 95:3191–3198. [PubMed: 16452253]
94. Zenisek D, et al. Transport, capture and exocytosis of single synaptic vesicles at active zones. *Nature.* 2000; 406:849–854. [PubMed: 10972279]
95. Li GL, et al. The unitary event underlying multiquantal EPSCs at a hair cell's ribbon synapse. *J Neurosci.* 2009; 29:7558–7568. [PubMed: 19515924]
96. Grant L, et al. Two modes of release shape the postsynaptic response at the inner hair cell ribbon synapse. *J Neurosci.* 2010; 30:4210–4220. [PubMed: 20335456]
97. Matthews G, Sterling P. Evidence that vesicles undergo compound fusion on the synaptic ribbon. *J Neurosci.* 2008; 28:5403–5411. [PubMed: 18495874]
98. 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]
99. Singer JH. Multivesicular release and saturation of glutamatergic signalling at retinal ribbon synapses. *J Physiol (Lond).* 2007; 580:23–29. [PubMed: 17218359]

100. Sagdullaev BT, et al. Presynaptic inhibition modulates spillover, creating distinct dynamic response ranges of sensory output. *Neuron*. 2006; 50:923–935. [PubMed: 16772173]
101. Zhang J, Diamond JS. Distinct perisynaptic and synaptic localization of NMDA and AMPA receptors on ganglion cells in rat retina. *J Comp Neurol*. 2006; 498:810–820. [PubMed: 16927255]
102. Matsui K, et al. Modulation of excitatory synaptic transmission by GABA(C) receptormediated feedback in the mouse inner retina. *J Neurophysiol*. 2001; 86:2285–2298. [PubMed: 11698519]
103. Vigh J, von Gersdorff H. Prolonged reciprocal signaling via NMDA and GABA receptors at a retinal ribbon synapse. *J Neurosci*. 2005; 25:11412–11423. [PubMed: 16339035]
104. Stevens CF, Wang Y. Facilitation and depression at single central synapses. *Neuron*. 1995; 14:795–802. [PubMed: 7718241]
105. Clements JD. Transmitter timecourse in the synaptic cleft: its role in central synaptic function. *Trends Neurosci*. 1996; 19:163–171. [PubMed: 8723198]
106. Rosenmund C, et al. The tetrameric structure of a glutamate receptor channel. *Science*. 1998; 280:1596–1599. [PubMed: 9616121]
107. Ricci-Tersenghi F, et al. Multivesicular release at developing Schaffer collateral-CA1 synapses: an analytic approach to describe experimental data. *J Neurophysiol*. 2006; 96:15–26. [PubMed: 16598063]
108. Smejkalova T, Woolley CS. Estradiol acutely potentiates hippocampal excitatory synaptic transmission through a presynaptic mechanism. *J Neurosci*. 2010; 30:16137–16148. [PubMed: 21123560]
109. Bolshakov VY, et al. Recruitment of new sites of synaptic transmission during the cAMP-dependent late phase of LTP at CA3-CA1 synapses in the hippocampus. *Neuron*. 1997; 19:635–651. [PubMed: 9331354]
110. Buhl EH, et al. Effect, number and location of synapses made by single pyramidal cells onto aspiny interneurons of cat visual cortex. *J Physiol*. 1997; 500(Pt 3):689–713. [PubMed: 9161986]
111. Egger V, et al. Coincidence detection and changes of synaptic efficacy in spiny stellate neurons in rat barrel cortex. *Nat Neurosci*. 1999; 2:1098–1105. [PubMed: 10570487]
112. Gabernet L, et al. Somatosensory integration controlled by dynamic thalamocortical feed-forward inhibition. *Neuron*. 2005; 48:315–327. [PubMed: 16242411]
113. Bruno RM, Sakmann B. Cortex is driven by weak but synchronously active thalamocortical synapses. *Science*. 2006; 312:1622–1627. [PubMed: 16778049]
114. Bagnall MW, et al. Multiple clusters of release sites formed by individual thalamic afferents onto cortical interneurons ensure reliable transmission. *Neuron*. 2011; 71:180–194. [PubMed: 21745647]
115. Chalifoux JR, Carter AG. GABAB receptors modulate NMDA receptor calcium signals in dendritic spines. *Neuron*. 2010; 66:101–113. [PubMed: 20399732]
116. Eccles JC, et al. The excitatory synaptic action of climbing fibres on the purinje cells of the cerebellum. *J Physiol (Lond)*. 1966; 182:268–296. [PubMed: 5944665]
117. Konnerth A, et al. Synaptic currents in cerebellar Purkinje cells. *Proc Natl Acad Sci USA*. 1990; 87:2662–2665. [PubMed: 1969639]
118. Perkel DJ, et al. Excitatory synaptic currents in Purkinje cells. *Proc Biol Sci*. 1990; 241:116–121. [PubMed: 1978337]
119. Foster KA, Regehr WG. Variance-mean analysis in the presence of a rapid antagonist indicates vesicle depletion underlies depression at the climbing fiber synapse. *Neuron*. 2004; 43:119–131. [PubMed: 15233922]
120. Armstrong DM, Rawson JA. Activity patterns of cerebellar cortical neurones and climbing fibre afferents in the awake cat. *J Physiol (Lond)*. 1979; 289:425–448. [PubMed: 458677]
121. Foster KA, et al. Interaction of postsynaptic receptor saturation with presynaptic mechanisms produces a reliable synapse. *Neuron*. 2002; 36:1115–1126. [PubMed: 12495626]

122. Takahashi M, et al. Pre- and postsynaptic determinants of EPSC waveform at cerebellar climbing fiber and parallel fiber to Purkinje cell synapses. *J Neurosci.* 1995; 15:5693–5702. [PubMed: 7643211]
123. Carey MR, Regehr WG. Noradrenergic control of associative synaptic plasticity by selective modulation of instructive signals. *Neuron.* 2009; 62:112–122. [PubMed: 19376071]
124. Bloedel JR, et al. Increased responsiveness of Purkinje cells associated with climbing fiber inputs to neighboring neurons. *J Neurophysiol.* 1983; 50:220–239. [PubMed: 6308181]
125. Jorntell H, Ekerot CF. Receptive field plasticity profoundly alters the cutaneous parallel fiber synaptic input to cerebellar interneurons in vivo. *J Neurosci.* 2003; 23:9620–9631. [PubMed: 14573542]
126. Trigo FF, et al. Readily releasable pool of synaptic vesicles measured at single synaptic contacts. *Proc Natl Acad Sci U S A.* 2012; 109:18138–18143. [PubMed: 23074252]
127. Crowley JJ, et al. Fast vesicle replenishment and rapid recovery from desensitization at a single synaptic release site. *J Neurosci.* 2007; 27:5448–5460. [PubMed: 17507567]
128. Satake S, et al. Paired-pulse facilitation of multivesicular release and intersynaptic spillover of glutamate at rat cerebellar granule cell-interneuron synapses. *J Physiol.* 2012; 590:5653–5675. [PubMed: 22930264]
129. Zhang W, Linden DJ. Neuromodulation at single presynaptic boutons of cerebellar parallel fibers is determined by bouton size and basal action potential-evoked Ca transient amplitude. *J Neurosci.* 2009; 29:15586–15594. [PubMed: 20007482]
130. Zhang W, Linden DJ. Calcium influx measured at single presynaptic boutons of cerebellar granule cell ascending axons and parallel fibers. *Cerebellum.* 2012; 11:121–131. [PubMed: 20049574]
131. Karakossian MH, Otis TS. Excitation of cerebellar interneurons by group I metabotropic glutamate receptors. *J Neurophysiol.* 2004; 92:1558–1565. [PubMed: 15152021]
132. Beierlein M, Regehr WG. Brief bursts of parallel fiber activity trigger calcium signals in bergmann glia. *J Neurosci.* 2006; 26:6958–6967. [PubMed: 16807325]
133. Tsai MC, et al. Neuronal glutamate transporters regulate glial excitatory transmission. *J Neurosci.* 2012; 32:1528–1535. Q9. [PubMed: 22302796]
134. Borst JG, Soria van Hoeve J. The calyx of held synapse: from model synapse to auditory relay. *Annu Rev Physiol.* 2012; 74:199–224. [PubMed: 22035348]
135. 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. [PubMed: 11124994]
136. Taschenberger H, et al. Optimizing synaptic architecture and efficiency for high-frequency transmission. *Neuron.* 2002; 36:1127–1143. [PubMed: 12495627]
137. Hashimoto K, Kano M. Functional differentiation of multiple climbing fiber inputs during synapse elimination in the developing cerebellum. *Neuron.* 2003; 38:785–796. [PubMed: 12797962]
138. Hashimoto K, et al. Translocation of a "winner" climbing fiber to the Purkinje cell dendrite and subsequent elimination of "losers" from the soma in developing cerebellum. *Neuron.* 2009; 63:106–118. [PubMed: 19607796]
139. Armario A, et al. Long-term neuroendocrine and behavioural effects of a single exposure to stress in adult animals. *Neuroscience and biobehavioral reviews.* 2008; 32:1121–1135. [PubMed: 18514314]
140. Gordon GR, Bains JS. Noradrenaline triggers multivesicular release at glutamatergic synapses in the hypothalamus. *J Neurosci.* 2005; 25:11385–11395. [PubMed: 16339033]
141. Bains JS, Ferguson AV. Nitric oxide regulates NMDA-driven GABAergic inputs to type I neurones of the rat paraventricular nucleus. *J Physiol.* 1997; 499(Pt 3):733–746. [PubMed: 9130169]
142. Quinlan ME, Hirasawa M. Multivesicular release underlies short term synaptic potentiation independent of release probability change in the supraoptic nucleus. *PLoS One.* 2013; 8:e77402. [PubMed: 24086774]

143. Clements JD, Silver RA. Unveiling synaptic plasticity: a new graphical and analytical approach. *Trends Neurosci.* 2000; 23:105–113. [PubMed: 10675910]
144. Clements JD. Variance-mean analysis: a simple and reliable approach for investigating synaptic transmission and modulation. *J Neurosci Methods.* 2003; 130:115–125. [PubMed: 14667541]
145. Reid CA, Clements JD. Postsynaptic expression of long-term potentiation in the rat dentate gyrus demonstrated by variance-mean analysis. *J Physiol (Lond).* 1999; 518:121–130. [PubMed: 10373694]
146. Meyer AC, et al. Estimation of quantal size and number of functional active zones at the calyx of held synapse by nonstationary EPSC variance analysis. *J Neurosci.* 2001; 21:7889–7900. [PubMed: 11588162]
147. 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]
148. Saviane C, Silver RA. Errors in the estimation of the variance: implications for multiple-probability fluctuation analysis. *J Neurosci Methods.* 2006; 153:250–260. [PubMed: 16376992]
149. Ikeda K, et al. Distinctive quantal properties of neurotransmission at excitatory and inhibitory autapses revealed using variance-mean analysis. *J Neurosci.* 2008; 28:13563–13573. [PubMed: 19074030]
150. Arancio O, et al. Excitatory synaptic connections onto rat hippocampal inhibitory cells may involve a single transmitter release site. *J Physiol.* 1994; 481(Pt 2):395–405. [PubMed: 7738832]
151. Wall MJ, Usowicz MM. Development of the quantal properties of evoked and spontaneous synaptic currents at a brain synapse. *Nat Neurosci.* 1998; 1:675–682. [PubMed: 10196583]
152. Franks KM, et al. Independent sources of quantal variability at single glutamatergic synapses. *J Neurosci.* 2003; 23:3186–3195. [PubMed: 12716926]
153. Laake JH, et al. Glutamine from glial cells is essential for the maintenance of the nerve terminal pool of glutamate: immunogold evidence from hippocampal slice cultures. *J Neurochem.* 1995; 65:871–881. [PubMed: 7616248]
154. Clements JD, et al. The time course of glutamate in the synaptic cleft. *Science.* 1992; 258:1498–1501. [PubMed: 1359647]
155. Kullmann DM, Asztely F. Extrasynaptic glutamate spillover in the hippocampus: evidence and implications. *Trends Neurosci.* 1998; 21:8–14. [PubMed: 9464678]
156. Pickett JA, Edwardson JM. Compound exocytosis: mechanisms and functional significance. *Traffic.* 2006; 7:109–116. [PubMed: 16420520]
157. He L, et al. Compound vesicle fusion increases quantal size and potentiates synaptic transmission. *Nature.* 2009; 459:93–97. [PubMed: 19279571]
158. 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]
159. Takamori S, et al. Molecular anatomy of a trafficking organelle. *Cell.* 2006; 127:831–846. [PubMed: 17110340]
160. Choi S, et al. Postfusional regulation of cleft glutamate concentration during LTP at 'silent synapses'. *Nat Neurosci.* 2000; 3:330–336. [PubMed: 10725921]
161. Renger JJ, et al. A developmental switch in neurotransmitter flux enhances synaptic efficacy by affecting AMPA receptor activation. *Neuron.* 2001; 29:469–484. [PubMed: 11239436]
162. Schoch S, et al. RIM1 α forms a protein scaffold for regulating neurotransmitter release at the active zone. *Nature.* 2002; 415:321–326. [PubMed: 11797009]
163. Kaeser PS, et al. RIM proteins tether Ca²⁺ channels to presynaptic active zones via a direct PDZ-domain interaction. *Cell.* 2011; 144:282–295. [PubMed: 21241895]
164. Hoppa MB, et al. Multivesicular exocytosis in rat pancreatic beta cells. *Diabetologia.* 2012; 55:1001–1012. [PubMed: 22189485]
165. Sheng J, et al. Calcium-channel number critically influences synaptic strength and plasticity at the active zone. *Nat Neurosci.* 2012; 15:998–1006. [PubMed: 22683682]

- MVR enhances reliability of synaptic transmission throughout the brain
- MVR controls local synaptic integration and network function
- Modulation of MVR on various timescales increases the dynamic range of synapses

BOX 1**Detecting multivesicular release (related to Figure 2)**

Variance-mean analysis (VMA) determines the quantal parameters q , P_r , and N by relating synaptic current fluctuations (expressed as CV) to mean synaptic responses under various P_r conditions [143, 144] (Fig. 2Aiii). Together with anatomical reconstruction (Fig. 2Ai, 2Aii) and Gaussian histograms of synaptic response amplitudes, VMA serves to identify release modality [145, 146, 119, 147–149]. Narrow amplitude histograms with equally spaced peaks are thought to reflect release of several vesicles. Uniquantal models assume that peaks represent the number of release sites, each releasing *at most* one vesicle, while multiquantal models assume that peaks reflect release of multiple vesicles from a common active zone [11, 20]. Single-peak histograms are thought to correspond to single-site UVR contacts [4, 150], where synaptic variability, (e.g. electrotonic distance from recording site, vesicle size, transmitter content and receptor density) generate amplitude fluctuations [26, 10, 151] but see [28, 152, 29, 40]. However, amplitude fluctuations could also be consistent with saturation, spillover or desensitization during MVR.

MVR can be measured directly when the presynaptic neuron only forms a single contact with the postsynaptic neuron [13, 127]. Here, spontaneous PSCs have a significant fraction of double-peaked events that occur within short time intervals and statistical analysis suggests that double peaks appear more often than expected by chance. Therefore, double peaks likely emerge from multiple vesicles released from a common active zone that interact with the same postsynaptic receptor population.

To detect MVR across a population of synapses, a receptor antagonist with a fast unbinding rate (typically a ‘low affinity antagonist’) [153] is a powerful yet simple tool that takes advantage of the non-equilibrium interaction of neurotransmitter and antagonist with receptor binding sites [105]. Unlike a slowly unbinding, high-affinity antagonist that inhibits the PSC in an antagonist concentration-dependent manner (Fig. 2Bi), the extent of inhibition by the fast-off antagonist also depends on transmitter concentration if the antagonist dissociation rate is shorter than the presence of synaptically released transmitter. As a result both transmitter and antagonist compete for receptor binding sites until transmitter is cleared [154, 12] (Fig 2Bii). This allows the fast-off antagonist to distinguish between MVR and UVR because MVR results in higher transmitter concentrations in the synaptic cleft than UVR (Fig. 2Biii). The advantages of the fast-off antagonist are easy implementation, few assumptions on quantal parameters, number of release sites and synaptic inputs. However, it is unsuitable to report release heterogeneity between release sites and performs less reliably at closely spaced sites because spillover and MVR might become indistinguishable [155].

Optical quantal analysis monitors NMDAR-mediated calcium fluxes in response to vesicle fusion at individual presynaptic boutons, and requires the loading of the neuron with a calcium-sensitive fluorescent dye [15, 18]. Similar to quantal current, the fusion of a vesicle causes a quantal fluorescence change (Fig. 2Ci). Hence, a step-wise increase of fluorescence signals reflects an increase in the number of fusing vesicles, or MVR (Fig.

2Cii and 2Ciii). Another optical technique uses the pH-sensitive green fluorescent protein pHluorin coupled to a vesicular transporter (e.g. vGlutpHluorin) to monitor vesicle fusion at single sites and takes advantage of a pH-dependent increase in fluorescence that occurs when the acidified vesicle is exposed to the basic extracellular milieu [38].

BOX 2**A mechanism for MVR**

It is not known *how* MVR occurs. It appears most likely that many vesicles fuse independently with the plasma membrane (heterotypic fusion) at most synapses [13–15, 39]. But alternative explanations have been considered. One example is the fusion of multiple vesicles with each other prior to exocytosis (homotypic fusion or compound fusion). First observed in secretory cells [156], activity and calcium-dependent compound fusion that could explain seemingly coordinated MVR was also reported at ribbon synapses [97] and at the calyx of Held [157]. However, the conditions under which compound fusion could occur at conventional synapses remain unknown. Several lines of evidence argue against compound fusion as a general mechanism for MVR: at the ribbon MVR can persist even in the presence of fast calcium buffers [158, 21], MVR exhibits similar intra and intersite temporal jitter [39], and proteins of the vesicle release machinery located on the plasma membrane and synaptic vesicles are asymmetrically distributed [159]. These observations support the notion of multiple independent release events within a release site [13–15, 39]. In addition, fusion pore dynamics were hypothesized to govern peak transmitter concentration in the synaptic cleft. Two fusion modes were proposed: incomplete fusion (‘kiss and run’) that frees transmitter slowly and might prolong the transmitter transient; or rapid full fusion, resulting in high transmitter concentration [160, 161]. However, the lack of kinetic changes in mEPSC time course at varying P_r s argues against such a mechanism to explain fluctuations in transmitter concentration [17, 39]. In conclusion, the most likely factors in regulating the number of releasable vesicles, setting P_r and determining the temporal pattern of fusion that should be investigated in the context of MVR are density, recruitment and type of presynaptic calcium channels, calcium buffering proteins, as well as proteins of the presynaptic vesicle release machinery [162, 98, 163, 84, 164, 165].

Outstanding questions

- The molecular determinants of UVR and MVR are unknown; and the composition of active zones that support MVR need further elucidation. Important factors may include calcium channel density, subtypes and adapter proteins, calcium sensors of the release machinery, calcium buffering, availability of release sites, and trans-synaptically acting proteins.
- The detailed mechanisms that govern the temporal pattern of MVR (synchronized vs. desynchronized) are unresolved.
- Presynaptic signaling pathways that underlie activity-dependent regulation of MVR, especially during intermediate and short-term plasticity, need additional exploration.
- The consequences of MVR vs. UVR for synaptic integration at single spines and dendritic branches are not well understood. This includes understanding how MVR contributes to the duration of postsynaptic potential, recruitment of voltage-gated and calcium-dependent conductances
- Consequences of MVR for network function (spillover-mediated recruitment of inhibition, action potential propagation) have been described in detail in the cerebellum. Whether similar phenomena apply to other circuits is unknown.

Figure 1. Multiquantal release at single synapses

(Ai and Aii) Electron micrograph and corresponding cartoon of a hippocampal synapse imaged following rapid freezing. Arrows indicate the presence of three docked vesicles at the active zone.

(Bi) Electron micrograph of two synaptic vesicles fusing with the within an active zone simultaneously.

(Bii) Cartoon representation on (Bi) illustrating neurotransmitter released from MVR interacts with a common pool of postsynaptic receptors (green and orange). *Adapted from [22].*

(C) Overview of different release modalities and their consequences for postsynaptic currents. UVR causes a low-concentration, short-lived transmitter transient in the synaptic cleft that results in a small postsynaptic EPSC (left). Synchronous fusion of several vesicles, MVR, results in elevated synaptic transmitter concentration and a large postsynaptic EPSC (middle). Desynchronized MVR prolongs but reduces the transmitter concentration transient resulting in a smaller and slowed postsynaptic EPSC (right). *Adapted from [39].*

Figure 2. Detecting multivesicular release (MVR)

(Ai) Reconstruction from paired recordings of a presynaptic basket (soma and dendrites in black, axon red) and postsynaptic pyramidal cell (soma and dendrites in blue, axon gray). Inset, EM-identified boutons shown at higher magnification.

(Aii) Electron micrographs show synaptic contacts between the presynaptic basket terminals and the pyramidal cell soma. Arrowheads indicate synaptic junctions.

(Aiii) Presynaptic action potentials (red) and resulting currents (blue) recorded under low P_r (left) and high P_r (right) conditions. Variance versus mean plot from current responses recorded in (Aiii) was used to derive quantal parameters (bottom). *Adapted from [30].*

(Bi) Top: Slow-off, high-affinity antagonist (labeled as 'slow') occupies postsynaptic receptors (in orange). Bottom: As a result of slow antagonist unbinding, synaptically released neurotransmitter (NT) binds only to the fraction of receptors that is not bound by the antagonist.

(Bii) Top: Due to its fast off-rate a low-affinity antagonist (labeled as 'fast') rapidly unbinds from receptors. Bottom: Synaptically released transmitter competes with the antagonist for receptor binding sites. Therefore, inhibition by the antagonist depends on the transmitter concentration and time course.

(Biii) The slow high-affinity antagonist inhibits PSCs recorded under high and low P_r conditions to the same extent (left). The fast low-affinity antagonist inhibits the EPSC recorded under high P_r conditions to a lesser extent than at low P_r conditions (right). *Adapted from [39].*

(Ci) Raster image of a dendrite with spines (red fluorescence) and the calcium transient after synaptic stimulation (green fluorescence). White lines and arrows indicate position of the line scan and time of synaptic stimulation, respectively. Synaptic stimulation results in a rapid increase of the green fluorescence (calcium sensitive) that does not occur with the red fluorescence (calcium insensitive).

(Cii) The ratio of green/red fluorescence in response to a single (left) or a pair of synaptic stimuli (right) shown by arrows. Failures of synaptic transmission can be clearly distinguished from successes.

(Ciii) Average fluorescence responses to a single successful stimulus (yellow) and to the second stimulus in a pair when the first stimulation produced a failure (green). The failure to both stimuli is also shown (black). *Adapted from [15].*