The Synaptic Vesicle Cycle Revisited: New Insights into the Modes and Mechanisms

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Abstract

Neurotransmission is sustained by endocytosis and refilling of synaptic vesicles (SVs) locally within the presynapse. Until recently, a consensus formed that following exocytosis, SVs are recovered by either fusion pore closure (kiss-and-run) or clathrin-mediated endocytosis directly from the plasma membrane. However new research has revealed that SV formation is more complex than previously envisaged. For example, in ultrafast endocytosis and activity-dependent bulk endocytosis, two additional recycling pathways discovered, SVs regenerate from the internalized membrane and endosomes. Furthermore, these diverse modes of endocytosis appear to influence both the molecular composition and subsequent physiological role of individual SVs. In addition, previously unknown complexity in SV refilling and re-clustering has been revealed. This article presents a modern view of the SV life cycle and discusses how neuronal subtype, physiological temperature, and individual activity patterns can recruit different endocytosis modes to generate new SVs and sculpt subsequent presynaptic performance.

Introduction

For chemical neurotransmission, SVs fuse with the plasma membrane to release neurotransmitter. To support high rates of release, synapses require a constant supply of neurotransmitter-filled SVs. This supply is maintained largely through local endocytosis in nerve terminals and rapid refilling of newly formed SVs with neurotransmitter. This entire process of reconstituting SVs after fusion is referred to as SV recycling.

Since the 1970s, the mechanisms by which SVs are recycled and trafficked at synapses have been intensely contested. On one side, using the frog neuromuscular junction, Heuser and Reese reported that SVs are regenerated locally by the formation of clathrin-coated vesicles at the periphery of active zones (Heuser and Reese, 1973). Thus, they proposed that SV recycling occurs via clathrin-mediated endocytosis (CME) from the plasma membrane **(Fig. 1)**. Around the same time, using similar experimental conditions, Ceccarelli, Hurlbut and Mauro reported scant evidence of clathrin-coated vesicles at synapses, but instead reported clear, uncoated vesicles potentially being internalized at the active zone (Ceccarelli et al., 1973). They proposed that SVs can be recycled by the reversal of an exocytic fusion pore, a model that was later termed "kiss-and-run" **(Fig. 1)** (Fesce et al., 1994). Thus ensued a 40-year debate about how SVs are recycled and the underlying mechanisms.

In the intervening decades, these models were further tested as new molecular and imaging tools became available. However, instead of resolving the issue, two other models for SV recycling has emerged: activity-dependent bulk endocytosis (ADBE) and ultrafast endocytosis. It is possible that all four mechanisms co-exist in nerve terminals and are used differently depending on activity levels or synapse type (Gan and Watanabe, 2018). Adding to the complexity is that SVs are functionally heterogeneous, defined by distinct molecular compositions (Crawford and Kavalali, 2015). Thus, different recycling mechanisms may help to sort cargoes during SV re-formation, ensuring that the proper molecular identity is maintained (Morgan et al., 2013). Additionally, recent data have revealed mechanistic insights into how newly-endocytosed SVs are re-filled with neurotransmitter and subsequently re-clustered (Farsi et al., 2018; Milovanovic et al., 2018). In this review, we discuss these recent developments, and in doing so, present a modern view of the SV cycle – exocytosis, endocytosis, endosomal sorting, neurotransmitter refilling, and re-clustering of SVs. However, due to space limitation, this review will focus mainly on the recycling aspect of the SV cycle. For mechanisms of exocytosis, please refer to recent excellent reviews from our colleagues [].

Synaptic vesicle heterogeneity and functional pools

SVs fuse and release neurotransmitter either in a time-locked or delayed manner following action potentials. The time-locked, synchronous phase of release transmits fast and reliable signals, while delayed asynchronous release influences network parameters, including efficacy of neurotransmission, synchronicity and plasticity (Otsu et al., 2004; Iremonger and Bains, 2016; Luo and Sudhof, 2017). In addition, SVs can fuse spontaneously in absence of action potentials, potentially affecting synapse formation and the strength of the connection []. Although not apparent from electron micrographs, increasing evidence suggests that visually-identical SVs have different molecular compositions, and this heterogeneity may underlie functional organization of

vesicle pools that differentially participate in the three phases of release (Chanaday and Kavalali, 2018).

SVs are organized into four functional pools at presynapses: readily-releasable pool (RRP), recycling pool, reserve pool and resting pool. For synchronous neurotransmission, a subset of SVs are docked (physically in contact with the plasma membrane) and primed (molecularly engaged) in a fusion-ready state at the active zone (Hammarlund et al., 2007; Sudhof, 2013; Neher and Brose, 2018). Docked SVs, as well as those located near the active zone, constitute the RRP that is immediately available for fusion upon the arrival of action potentials (Holderith et al., 2012). To sustain neurotransmitter release, the RRP must be constantly replenished with SVs (Guo et al., 2015). This replenishment can be accomplished by either rapid re-use of fused vesicles or recruitment of new SVs from the "reserve pool" during prolonged periods of activity. Collectively, all SVs that participate in activity-induced synaptic transmission (RRP and a part of the reserve pool) comprise the recycling pool (Denker and Rizzoli, 2010), which is estimated to be ~50% of total SVs (Kim and Ryan, 2010), but may be as few as 1-5% percent (Denker et al., 2011). The remaining SVs may be referred to as the resting pool since they are reluctant to be mobilized after stimulation (Chanaday and Kavalali, 2018).

Within the recycling pool, distinct molecular machineries determine whether vesicles fuse synchronously or asynchronously. Synchronous fusion is mediated by the canonical neuronal Soluble NSF Attachment protein Receptor (SNARE) complex, which includes the SV proteins (cargos), synaptobrevin 2 (syb-2/VAMP2) (Jahn and Fasshauer, 2012; Rizo and Sudhof, 2012), and the neuronal calcium-sensing proteins synaptotagmins 1, 2 and 9. Synaptotagmins clamp the SNARE complex in the absence of an action potential and trigger synchronous fusion in response to local calcium entry through voltage-gated calcium channels (Sudhof, 2013). In contrast, asynchronous release is conferred by synaptotagmin 7 (Bacaj et al., 2013) or Doc2 (Yao et al., 2011), and by the non-canonical SNARE VAMP4 (Raingo et al., 2012). These molecular differences are likely maintained throughout the SV cycle. A recent report suggests that synaptotagmin 1 and 7 couple synchronous and asynchronous release to a fast (1-2 s) or slow (several seconds) mode of endocytosis, respectively (Li et al., 2017). Moreover, the asynchronous SNARE VAMP4 is required for ADBE after intense activity (Nicholson-Fish et al., 2015), during which asynchronous release becomes more prominent. Thus, current evidence suggests that synchronous and asynchronous release is maintained through different recycling pathways.

SVs can also spontaneously fuse and recycle in the absence of action potentials (Kavalali, 2015). Although it is debated whether spontaneous release draws from the recycling pool, resting pool, or its own pool (Sara et al., 2005; Fredj and Burrone, 2009), the presence of non-canonical SNAREs VAMP4, VAMP7, and Vti1a likely defines whether particular vesicles fuse spontaneously (Ramirez and

Kavalali, 2012; Bal et al., 2013; Chanaday and Kavalali, 2018). A number of calcium sensors have been proposed to trigger spontaneous neurotransmitter release, including the Doc2 family of proteins (Ramirez et al., 2017; Courtney et al., 2018). In addition to these molecular differences, endocytosis of spontaneously fused SVs occurs at a faster timescale (<1 s) and is partially calcium-independent (Leitz and Kavalali, 2014), implicating a distinct mode of endocytosis. Thus, several modes of endocytosis likely maintain vesicle supply at presynaptic terminals for distinct phases of neurotransmission.

Modes of synaptic vesicle recycling

At present, at least four modes of SV recycling have been identified, distinguished by their molecular mechanisms and speed: CME, kiss-and-run, ultrafast endocytosis and ADBE **(Fig. 2)**.

Clathrin-mediated endocytosis vs kiss-and-run

Over the last two decades, many studies focused on addressing whether SVs are recycled via CME and kiss-and-run. The kinetics and molecular requirements distinguish these two modes of endocytosis: CME is relatively slow (10-30 s) and requires a distinct set of molecules **(Fig. 2A)** (Saheki and De Camilli, 2012; Milosevic, 2018). In contrast, kiss-and-run is fast (<1-2 s) and does not require clathrin-associated proteins. Thus, these features have been investigated extensively at model synapses ranging from those in invertebrates, such as nematodes *(C. elegans)* (Nonet et al., 1999), fruit fly (Zhang et al., 1998; Heerssen et al., 2008), and squid (Morgan et al., 1999; Morgan et al., 2000; Morgan et al., 2001), to vertebrates, such as lampreys (Shupliakov et al., 1997; Walsh et al., 2018) and rodents (Granseth et al., 2006; Mani et al., 2007). At squid synapses, disrupting the functions of core clathrin coat components, such as adaptor proteins (AP180, AP-2) or clathrin uncoating proteins (Hsc70, auxilin) severely impaired neurotransmission, indicating an essential role for the clathrin pathway (Morgan et al., 1999; Morgan et al., 2000; Morgan et al., 2001). At mammalian nerve terminals, knock-down of clathrin-heavy chain (CHC) suggested that almost all endocytosis is clathrin-mediated (Granseth et al., 2006). A unified view from these studies is that SV recycling requires clathrin and clathrin-associated proteins, and, where measured, occurs slowly with a single kinetic component (Balaji and Ryan, 2007) [but see https://www.ncbi.nlm.nih.gov/pubmed/19217377]. These studies led to the idea that CME predominates in these synapses. For more complete reviews on molecular mechanisms, please refer to recent articles by our colleagues [].

Nonetheless, the essential role for CME in recycling of SVs from the plasma membrane and retrieval of SV cargos has been questioned. New data suggest that endocytosis can proceed after knockdown of CHC or its adaptor AP-2 (Kim and Ryan, 2009; Kononenko et al., 2014; Watanabe et al., 2014), pharmacological inhibition (Delvendahl et al., 2016), acute photo-inactivation

(Heerssen et al., 2008; Kasprowicz et al., 2008) or using temperature-sensitive CHC mutants (Yu et al., 2018), though in some cases compensatory endocytosis is aberrant and insufficient for regenerating SVs or sustaining neurotransmission (Heerssen et al., 2008; Kasprowicz et al., 2008). This lack of an obligatory requirement for CME was most prevalent at physiological temperatures in mammalian neurons (Watanabe et al., 2014; Delvendahl et al., 2016; Soykan et al., 2017). However, in all cases, clathrin is necessary during the SV cycle either at the plasma membrane, as previously understood, or from intracellular endosomes, as described below.

Ultrafast endocytosis

Another mode for SV recycling was recently identified, called "ultrafast endocytosis" **(Fig. 2A)**. Ultrafast endocytosis can complete in as fast as 50 ms following exocytosis and continues stochastically only for seconds (Watanabe et al., 2013d; Watanabe et al., 2013a; Delvendahl et al., 2016). This mode of SV recycling is predominant at physiological temperatures in both *C. elegans* neuromuscular junctions (NMJs, room temperature) and mouse hippocampal synapses (34-37 ºC). It occurs following brief neuronal activity, but may also operate during high-frequency stimulation (Watanabe et al., 2014; Soykan et al., 2017). In *C. elegans* NMJs and mouse central synapses, the lateral edges of an active zone mark sites of ultrafast endocytosis. Membrane at these sites rapidly invaginates to form a large endocytic vesicle (~80 nm) without the requirement for clathrin. These endocytic vesicles are delivered immediately to synaptic endosomes from which SVs are regenerated via budding in a clathrin-dependent manner (Watanabe et al., 2014). Membrane flux through exocytosis and ultrafast endocytosis is roughly equal. During trains of stimuli, ultrafast endocytosis is triggered multiple times to compensate for excess membrane added through SV fusion. It is worth noting that a form of clathrin-independent fast endocytosis has been observed in retinal bipolar neurons (von Gersdorff and Matthews, 1994), and ultrafast endocytosis shares many features with this pathway.

Because ultrafast endocytosis was discovered only recently, its molecular mechanism has not been explored extensively. However, several studies suggest that ultrafast endocytosis shares many molecular players with other endocytic pathways including CME. For example, synaptojanin-1 and endophilin-A, two key players in CME (Verstreken et al., 2003; Milosevic et al., 2011), coordinately tubulate the invaginated membrane at its base, forming a narrow neck on the budding vesicle (Watanabe et al., 2018). The vesicle is then pinched off at the neck by actions of the large GTPase, dynamin-1 (Watanabe et al., 2013d; Watanabe et al., 2013a). Polymerized actin is also essential in ultrafast endocytosis (Watanabe et al., 2013a), as it is during clathrin-dependent and clathrin-independent endocytosis at synapses (Shupliakov et al., 2002; Soykan et al., 2017). Theoretical and computational modeling studies suggest that ultrafast endocytosis relies on proper maintenance of membrane tension (Shi and Baumgart, 2015), which may be influenced by actin. Interestingly, ultrafast endocytosis fails completely under conditions where membrane fluidity is

reduced, for example by rapid cooling of cultured mouse neurons to room temperature (Watanabe et al., 2014). Further studies are required to elucidate the exact mechanism of ultrafast endocytosis.

Activity-dependent bulk endocytosis

In contrast to ultrafast endocytosis, longer bursts of intense activity trigger ADBE at invertebrate, amphibian, and mammalian synapses (Gan and Watanabe, 2018) and *in vivo* (Korber et al., 2012). ADBE retrieves large areas of membrane within 1-2 s to form intracellular endosomes (average ~150 nm) in a process that is clathrin-independent **(Fig. 2B)** (Clayton and Cousin, 2009; Kononenko and Haucke, 2015). This strict coupling of ADBE to neuronal activity is due to the transient activation of the calcium-dependent protein phosphatase calcineurin (Kokotos and Cousin, 2015). Recent studies have also highlighted a key role for the actin cytoskeleton in ADBE (Wu et al., 2016; Soykan et al., 2017). This suggests that a rapid, actin-dependent invagination drives formation of the bulk endosome, which may be coupled to neuronal activity by altered membrane tension during SV fusion events **(Fig. 2B)**. Inhibition of ADBE results in a modest relief of short-term depression (Clayton et al., 2010; Smillie et al., 2013), potentially by increasing the efficiency of SV cargo capture at the periactive zone. However, ADBE inhibition results in a reduced capacity to sustain neurotransmitter release in the longer term (Nicholson-Fish et al., 2015). When one considers the scope for its bidirectional modulation (Smillie et al., 2013; Kokotos et al., 2018), this suggests that ADBE provides a plastic, scalable mechanism to alter neuronal output.

Typical SV proteins (cargoes) such as VAMP2, synaptophysin, vesicular glutamate transporter (v-Glut) are retrieved by ADBE (Nicholson-Fish et al., 2015; Kokotos et al., 2018), though it is unclear whether this retrieval is direct or due to escape of excess cargo from saturated clustering mechanisms at the periactive zone. However, some cargoes, such as VAMP4, are preferentially accumulated by ADBE, perhaps explaining why VAMP4 is also essential for this mode of endocytosis (Nicholson-Fish et al., 2015). Interestingly, the SV calcium channel Flower, which is deposited into the plasma membrane during high activity, may provide calcium influx to trigger ADBE and thus facilitate the coupling of neuronal activity to ADBE (Yao et al., 2009; Yao et al., 2017). Therefore, specific proteins may play direct roles in ADBE, rather than being passively retrieved.

After ADBE, subsequent SV budding from internalized memebrae requires the efflux of previously accumulated extracellular calcium, which is driven by endosomal acidification (Cheung and Cousin, 2013). Cargo selection most likely occurs at this step, since both the classical plasma membrane adaptor AP-2 and endosomal AP-1/AP-3 are required for SV generation from bulk endosomes (Kononenko et al., 2014; Kokotos and Cousin, 2015). Since endophilin-dependent recruitment of synaptojanin-1 is determined by membrane curvature

(Chang-Ileto et al., 2011; Milosevic et al., 2011), this hybrid requirement for adaptors during ADBE may arise from heterogeneity in bulk endosome size (range: 100-500 nm). With larger bulk endosomes, which have shallower membrane curvature, endophilin and synaptojanin-1 recruitment would be inefficient, resulting in stabilized $PI(4,5)P_2$ and therefore enhanced AP-2 dependent cargo sorting, whereas smaller endosomes may utilize AP-1/AP-3. Consequently, the requirement of different adaptor proteins may result in SVs with varying molecular compositions, resulting in the functional heterogeneity discussed above (Silm et al., 2019).

The current view of synaptic vesicle recycling

To revisit the issue regarding the role of clathrin in SV recycling, decades of research implicate clathrin as an essential player in the regeneration of SVs. However, the location of these events may be dictated by stimulus intensity, temperature, and synapse type. In general, current data suggest that during lower activity levels and at temperatures significantly lower than physiological temperature most endocytic events are clathrin-mediated, since ADBE is inactive and ultrafast endocytosis is highly temperature-sensitive **(Fig. 2A**). At near-physiological temperature, regardless of stimulation, nascent plasma membrane sites of clathrin-mediated budding may be relocated to rapidly forming endosomes, although exceptions do exist. For example, squid and lampreys, which live at cooler temperatures (4-25 °C), may use CME exclusively for recycling SVs (Gad et al., 1998; Morgan et al., 2000). Thus, SV recycling might have evolved to adapt to changes in activity and environmental conditions.

The essential requirement for clathrin during SV re-formation may underscore why mutations or alterations in the levels of several clathrin-associated proteins are linked to neurodegeneration. These include deficiency in membrane curvature protein, endophilin-A, which is linked to age-dependent ataxia (Murdoch et al., 2016), as well as mutations in phosphoinositide phosphatase synaptojanin-1 and putative tyrosine-protein phosphatase auxilin, which are linked to inherited forms of Parkinson's disease or parkinsonism (Edvardson et al., 2012; Krebs et al., 2013). Similarly, a selective reduction of the clathrin adaptors AP180 and AP-2 has been reported in Alzheimer's disease (Yao and Coleman, 1998). Thus there are numerous links between defects in the clathrin pathway and neurodegenerative diseases.

With several new endocytic models revealed, the debate on SV recycling mechanisms is far from being resolved (Wu et al., 2014). Under all conditions discussed above, additional roles for kiss-and-run cannot be ruled out. The presence of kiss-and-run is well established in non-neuronal secretory cells (Ales et al., 1999; Burgoyne et al., 2001). Although scarcer, several optical approaches also indicate its existence at mammalian central synapses (Stevens and Williams, 2000; Zhang et al., 2009; Chanaday and Kavalali, 2018). Given the modulatory nature of SV cycling, it would be important to understand at what

stimulation frequency and temperature kiss-and-run is prevalent and which molecules stabilize the rapidly expanding fusion pore. With the refinement of tools and approaches, more data will likely arise in coming years.

Mechanisms of synaptic vesicle (re)acidification and (re)filling

After endocytosis and vesicle re-formation, newly formed SVs must be refilled with neurotransmitter and made fusion-ready (Blakely and Edwards, 2012; Farsi et al., 2017). Regardless of the mechanism of vesicle reformation, each SV must be rapidly loaded with more than a thousand neurotransmitter molecules (Riveros et al., 1986; Burger et al., 1989). The key components that execute neurotransmitter filling are the vacuolar H^+-ATPase (vATPase) and the vesicular neurotransmitter transporters. The evolutionarily conserved vATPase is a large multi-protein complex that consists of an integral V_0 domain, which translocates protons across the membrane, and a peripheral V_1 domain responsible for ATP hydrolysis (Stevens and Forgac, 1997; Toei et al., 2010). The vesicular neurotransmitter transporters determine neurotransmitter content (Gronborg et al., 2010). These two groups of proteins mediate distinct processes: the vATPase rapidly forms an electrochemical gradient ($\Delta\mu_{H+}$) across the membrane by pumping protons into the lumen of SVs with sub-second kinetics, whereas the transporters utilize this gradient to shuttle the neurotransmitter molecules into the SVs, although the exact loading mechanism differs depending on the charge of particular neurotransmitters (Blakely and Edwards, 2012). Nonetheless, under physiological conditions where ATP and neurotransmitter are abundant and readily available, these two processes likely occur in parallel.

The recycling of vesicular ATPases and neurotransmitter transporters must be tightly coupled with SV recycling. SVs isolated from mammalian brain contain many tens of copies of vesicular transporters, but only 1-2 copies of the vATPase (Takamori et al., 2006). Thus, the recycling process must ensure that at least one copy of the vATPase is sorted into each SV. In addition, recycled SVs must contain a proper set of transporters, particularly when more than one type of neurotransmitter transporters are available in the same neurons (i.e. vesicular monoamine and glutamate transporters). Sorting of these proteins requires clathrin and multiple adaptor protein complexes (AP1, AP2, and AP3) (Onoa et al., 2010; Blakely and Edwards, 2012; Silm et al., 2019), again suggesting the seential roles of clathrin-mediated process in SV recycling.

In addition to proper sorting of these proteins into SVs, clathrin likely plays an essential role in determining the timing of acidification and thereby neurotransmitter loading. A recent study suggests that reacidification of SVs relies on removal of clathrin-coats from vesicles, due to sterically hindering of vATPase by clathrin cages (Farsi et al., 2018). Upon uncoating, vesicles rapidly acidifies, suggesting that the removal of clathrin-coats ensures that neurotransmitter is loaded when SVs are reformed. Although partially filled SVs

are fusion-competent, incompletely filled vesicles have a lower release probability (Rost et al., 2015). Thus, by ensuring proper loading of neurotransmitter into vesicles, fidelity of neurotransmission is likely maintained.

Synaptic vesicle "maturation" and clustering

Finally, new SVs are captured into discrete SV clusters. During prolonged stimulation, vesicles are mobilized from these clusters to ensure continued neurotransmitter release. The primary components for vesicle clustering are the synapsins, which are highly abundant phosphoproteins that reversibly associate with SVs (De Camilli et al., 1983; Chi et al., 2001). Synapsins maintain the reserve pool via phosphorylation-dependent interactions with SVs and the actin cytoskeleton (Pieribone et al., 1995; Bloom et al., 2003; Gitler et al., 2008). Synapsins also functionally interact with α-synuclein (Atias et al., 2019), peripheral Rab3 proteins (Giovedi et al., 2004), and other Rab GTPases and their interactors (Pavlos and Jahn, 2011), to regulate SV clustering. Importantly, loss of function of synapsins is associated with a number of neurological and neuropsychiatric disorders, including autism, schizophrenia, and epilepsy (Garcia et al., 2004; Porton et al., 2011; Greco et al., 2013).

One critical aspect of vesicle clustering that has remained unclear is how all these proteins keep SVs clustered together while still allowing vesicle mobility. A recent study suggested that SV clusters represent an example of liquid condensates – distinct phase of liquid in aqueous environment, where lipid vesicles are captured by proteins of the interweaving matrix (Milovanovic and De Camilli, 2017). Indeed, synapsin was shown to organize vesicles in clusters *in vitro* by liquid-liquid phase separation, thereby suggesting that SV clustering at the presynaptic terminal can be explained at least in part by the phase separation principle (Milovanovic et al., 2018). In addition, some endocytic proteins, including amphiphysin, dynamin-1, and intersectin-1, have been found amongst the matrix components connecting SVs at resting state (Shupliakov et al., 2011), raising the possibility that the SV cluster may additionally provide a source for proteins involved in vesicle recycling. Upon stimulation, these endocytic proteins translocate to the periactive zone, thus coupling the processes of exocytosis and endocytosis (Evergren et al., 2004).

Conclusions

In summary, we now realize that the SV cycle is much more complex than previously thought. Given how important neurotransmission is to survival, in hindsight, it may not be so surprising that synapses harbor multiple modes of SV exocytosis and endocytosis to ensure their fidelity despite differences in activity levels and physiological temperatures, and to accommodate different release modes or synapse types. In cold-blooded animals, for example, the modes of SV recycling may shift seasonally as the animals adapt to environmental changes in

temperature. Emerging evidence also suggests that the different modes of vesicle recycling may supply SVs that are “tuned” (in molecular terms) to the function of the neuron. This might be especially important at synapses with phasic versus tonic activity, or with different rates of spontaneous release, or at sensory synapses that require particularly fast forms of neurotransmission.

Given this new knowledge, it will become increasingly important to measure SV recycling under experimental conditions that best mimic the synapses’ normal physiology or, in cases where this is not known, across different temperatures and stimulation intensities. Likewise, as we go forward in different model systems, it is essential to determine when and where the clathrin machinery acts during SV recycling. Such studies may reveal a molecular convergence between the different vesicle retrieval modes, or conversely highlight specific presynaptic adaptations driven by the variables listed above. Given the rapidly changing field, there are likely to be additional significant advances in the coming years that further illuminate the regulatory mechanisms of SV cycling and how they play together to ensure ongoing neurotransmission.

FIGURE LEGENDS

Figure 1. Classical view of the SV cycle. Action potentials trigger fusion of SVs at the active zone. After formation of the fusion pore, resulting in neurotransmitter release, two options are possible: the pore can re-close via kiss-and-run (K&V) or it can expand irreversibly leading to full-collapse. Compensating for full-collapse fusion, specific SV proteins are recruited by adaptor proteins at the periactive zone, triggering clathrin-mediated endocytosis (CME). Dynamin mediates vesicle scission, after which SVs are uncoated and refilled with neurotransmitters before being returned to the vesicle cluster.

Figure 2. Modern view of the SV cycle. A. During low activity levels, SVs are recruited to the RRP from the reserve/resting pool and fuse at the active zone, after which they may be retrieved via one of several mechanisms: 1) the fusion pore may reclose by kiss-and-run, 2) ultrafast endocytosis at the periactive zone can retrieve endocytic vesicles that rapidly fuse with synaptic endosomes from which SVs regenerate in a clathrin-dependent manner; 3) CME can generate SVs from the plasma membrane in certain circumstances, after which vesicle uncoating is necessary for the vATPase to acidify the lumen triggering concurrent neurotransmitter (NT) refilling by transporters. After refilling, some SVs are recruited back to the cluster, where they are segregated into functional pools. **B.** At high activity levels, many SVs are mobilized and exocytosed by full-collapse fusion. This activates ADBE, which retrieves large areas of membrane generating bulk endosomes from which SVs regenerate.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Dragomir Milovanovic for helpful comments on the manuscript. This work was supported by: Schram-Stiftung T287/25457 and Deutsche Forschungsgemeinschaft (Emmy Noether Young Investigator Award MI-1702/1) to IM; The Wellcome Trust (204954/Z/16/Z) to MAC; the National Science Foundation (1727260), the National Institutes of Health (NINDS DP2 NS111133 and R01 NS105810), the McKnight Foundation, the Sloan Foundation to SW; and the National Institutes of Health (NINDS/NIA R01 NS078165 to JRM and NIMH R01 MH066198 to Dr. Ege Kavalali which supports NC).

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