Neurotransmission is sustained by endocytosis and refilling of synaptic vesicles (SVs) locally within the presynapse. Until recently, a consensus formed that after exocytosis, SVs are recovered by either fusion pore closure (kiss-and-run) or clathrin-mediated endocytosis directly from the plasma membrane. However, recent data have revealed that SV formation is more complex than previously envisaged. For example, two additional recycling pathways have been discovered, ultrafast endocytosis and activity-dependent bulk endocytosis, in which SVs are regenerated from the internalized membrane and synaptic 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 reclustering has been revealed. This review presents a modern view of the SV life cycle and discusses how neuronal subtype, physiological temperature, and individual activity patterns can recruit different endocytic modes to generate new SVs and sculpt subsequent presynaptic performance.

Introduction

During chemical neurotransmission, synaptic vesicles (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 primarily through membrane endocytosis, cargo sorting, and rapid refilling of newly formed SVs with neurotransmitter, which occur locally within nerve terminals. This entire process of reconstituting SVs after fusion is referred to as SV “recycling.” In contrast, de novo synthesis of SVs in the cell body and axonal transport to synapses is usually too slow to support the high demand for neurotransmitter release. This review will focus mainly on the recycling aspect of the SV cycle. For mechanisms of exocytosis, please refer to recent excellent reviews (Rizo and Xu, 2015; Chanaday and Kavalali, 2018a; Neher and Brose, 2018; Brunger et al., 2019; Dittman and Ryan, 2019).

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 (1973) reported that SVs are regenerated locally by the formation of clathrin-coated vesicles at the periphery of active zones. 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 et al. (1973) reported scant evidence of clathrin-coated vesicles at synapses, but instead reported clear, uncoated vesicles potentially being internalized at the active zone. 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 have 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 reformation, 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 refilled with neurotransmitter and subsequently reclustered (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 with a specific emphasis on SV recycling mechanisms.
SV heterogeneity and functional pools

After action potentials, SVs fuse and release neurotransmitter either in a time-locked or delayed manner. 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 Südhof, 2017). In addition, SVs can fuse spontaneously in the absence of action potentials, potentially affecting synapse formation and the strength of the connection (Chanaday and Kavalali, 2018a). 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, 2018a).

SVs are organized into four functional pools at presynapses: the 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 (ready-to-fuse upon calcium elevation) in a fusion-ready state at the active zone (Hammarlund et al., 2007; Südhof, 2013; Neher and Brose, 2018). Docked and primed SVs, 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) (Jonas, 2014). This replenishment can be accomplished by either rapid reuse 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 comprise the recycling pool (i.e., RRP and a fraction of the reserve 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% (Denker et al., 2011). The remaining SVs may be referred to as the resting pool because they are reluctant to be mobilized during/after stimulation (Chanaday and Kavalali, 2018a).

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 protein synaptobrevin 2 (syb-2/VAMP2) (Jahn and Filshauer, 2012; Rizo and Südhof, 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 (Südhof, 2013). In contrast, asynchronous release is conferred by synaptotagmin 7 (Bacal et al., 2015) or Doc2 (Yao et al., 2011) and by the noncanonical SNARE VAMP4 (Rainingo 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 SV 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 noncanonical SNAREs VAMP4, VAMP7, and Vti1a likely defines whether particular vesicles fuse spontaneously (Ramirez and Kavalali, 2012; Bal et al., 2013; Chanaday and Kavalali, 2018a). 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 may maintain the SV supply at presynaptic terminals to support distinct phases of neurotransmission.

Modes of SV 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).

CME versus kiss-and-run

Over the last two decades, many studies have focused on addressing whether SVs are recycled via CME or 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, 2000, 2001), to vertebrates such as lampreys (Shaplikov et al., 1997; Walsh et al., 2018) and rodents (Gansseth 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-uncoupling proteins (Hsc70, auxillin) severely impaired neurotransmission, indicating an essential role for the clathrin pathway (Morgan et al., 1999, 2000, 2001). At mammalian nerve terminals, knock-down of clathrin-heavy chain suggested that almost all endocytosis is clathrin mediated (Gansseth 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 Zhu et al., 2009). These studies led to the idea that CME predominates in these synapses. For more complete reviews on molecular mechanisms of CME, please refer to recent articles (Saheki and De Camilli, 2012; Milosevic, 2018).

Nonetheless, the essential role for CME in recycling of SVs from the plasma membrane and retrieval of SV cargos has been questioned. More recent data suggest that endocytosis can proceed after knockdown of clathrin heavy chain 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 clathrin heavy chain mutants (Yu et al., 2018), though in some cases compensatory endocytosis was 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 called “ultrafast endocytosis” was recently identified (Fig. 2A). Ultrafast endocytosis can complete in as fast as 50 ms after exocytosis and continues stochastically only for seconds (Watanabe et al., 2013a,b; 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°C). It occurs after 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 approximately 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 mechanisms have not been explored as extensively as other modes of SV recycling. However, several studies suggest that ultrafast endocytosis shares many molecular players with other endocytic pathways including CME. For example, synaptopoatin-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 the actions of a large GTPase, dynamin-1 (Watanabe et al., 2013a,b). 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.

![Diagram](image-url)

**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, or (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, SVs can be 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.
Activity-dependent bulk endocytosis

In contrast to ultrafast endocytosis, longer bursts of intense activity trigger ADBE at invertebrate, amphibian, and mammalian synapses (Clayton et al., 2008; Gan and Watanabe, 2018) and in vivo (Köber 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 Haucek, 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 periaxonal 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, and 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 periaxonal zone. 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-associated 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, 2017). Therefore, specific vesicle proteins may play direct roles in ADBE rather than being passively retrieved.

After ADBE, subsequent SV budding from internalized membrane 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). Because 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 Pif(4,5)P2 and therefore enhanced AP-2-dependent cargo sorting, whereas smaller endosomes may use 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).

Current view of SV recycling

Although decades of research implicate clathrin as an essential player in the regeneration of SVs, 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 reformation may underscore why mutations or alterations in the levels of several well-characterized clathrin-associated proteins are linked to neurodegeneration. These include deficiency in membrane curvature sensing 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 (Alès 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, 2018b). 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, a better understanding of these processes will likely arise in coming years.

Mechanisms of SV (re)acidification and (re)filling

After endocytosis and vesicle reformation, 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 multiprotein complex that consists of an integral V0 domain, which translocates protons across the membrane, and a peripheral V1 domain responsible for ATP hydrolysis (Stevens and Forcag, 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 (ΔμH+) across the membrane by pumping protons into the lumen of SVs with subsecond
kinetics, whereas the transporters use 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.

Each SV isolated from mammalian brain contains many tens of copies of vesicular transporters, but only one or two copies of the vATPase (Takamori et al., 2006). The recycling of vATPases and neurotransmitter transporters must therefore be tightly coupled with SV recycling, and at least one copy of the vATPase must be sorted into each SV to allow subsequent neurotransmitter loading in the vesicle. In addition, recycled SVs should 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 transporters requires clathrin and multiple adaptor protein complexes (AP1, AP2, and AP3) (Ono et al., 2010; Blakely and Edwards, 2012; Slim et al., 2019), again pointing to the essential roles of clathrin-mediated processes in SV recycling.

In addition to proper sorting of SV proteins, clathrin likely plays an essential role in determining the timing of vesicle acidification and thereby neurotransmitter loading. A recent study suggests that reacidification of SVs relies on removal of clathrin-coats from vesicles, due to steric hindrance of the vATPase by clathrin cages (Farsi et al., 2018). Upon uncoating, vesicles rapidly acidify, suggesting that the removal of clathrin-coats ensures that neurotransmitter is loaded as soon as SVs are reformulated. 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 maintained.

SV “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; Gitter 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 interactions (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 among 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, it is now recognized 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.

References

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