Cycling the Synapse: Scenic versus Direct Routes for Vesicles

Gary Matthews*
Department of Neurobiology and Behavior
State University of New York at Stony Brook
Stony Brook, New York 11794

What happens to synaptic vesicles after they release their neurotransmitter content? Recent work on a variety of synaptic systems shows that there is no single answer to this question. Rather, it seems that neurons use a variety of methods to retrieve and reuse synaptic vesicles after they have undergone exocytosis. The challenge now is to establish the molecular mechanisms and to decipher the rules that govern which cycling pathway is used in a given functional context.

The synaptic vesicle cycle—exocytosis, retrieval, and reformation of new release-competent vesicles—is required to maintain release-ready vesicles during sustained synaptic activity. From the viewpoint of cell biology, this synaptic cycle is a special case of the more general cellular process of membrane trafficking, for which multiple mechanisms exist. But which of these mechanisms is used to separate the vesicle membrane from the plasma membrane after exocytosis? How are new vesicles generated after the membrane is retrieved? Several recent studies combining electrophysiology, fluorescence imaging, and electron microscopy have revealed more than one answer to these questions, depending on the type of synapse and the stimulation conditions. Given the importance of the vesicle cycle in neuronal function, it is perhaps not surprising that neurons tap more than one trafficking method to recycle vesicles during synaptic activity.

An Overview of the Vesicle Cycle

Figure 1 summarizes three alternative pathways for vesicle cycling: kiss-and-run exocytosis (pathway A, green arrows), full fusion followed by clathrin-dependent endocytosis (pathway B, red arrows), or full fusion followed by bulk membrane retrieval (pathway C, blue arrows). The latter two mechanisms require further processing within the terminal to generate new vesicles, either by direct uncoating of clathrin-coated vesicles or via intermediate endosomes (cisternae) that later give rise to new vesicles (Heuser and Reese, 1973). In the kiss-and-run scheme (Ceccarelli et al., 1973), no additional steps are necessary, because the vesicle membrane never merges with the plasma membrane.

Synaptic terminals contain from dozens to hundreds of thousands of vesicles, but not all of these vesicles are equal. A special subset—typically 10%–40% of the total—are released more readily and recycle more rapidly (green vesicles in Figure 1). As long as recycling of these vesicles can keep up with demand, neurotransmitter release is maintained largely by repeated cycling of this subset (e.g., Richards et al., 2003; Fernández-....

*Correspondence: gary.g.matthews@sunysb.edu

Minireview

Alfonso and Ryan, 2004). The reserve vesicles (yellow vesicles in Figure 1) come into play when demand exceeds the capacity of the rapidly cycling cohort. On the face of it, the kiss-and-run scheme seems particularly well-suited to such repeated cycling of a limited pool of vesicles. During kiss-and-run exocytosis, vesicles release neurotransmitter through a narrow pore that transiently connects the vesicle interior to the extracellular space. Once the pore closes, the empty vesicle either detaches from the active zone or it remains in place and refills with neurotransmitter for another round of release (kiss-and-stay). In either case, recently recycled vesicles occupy a privileged location near the active zone, which would neatly explain their preferential reuse. Is there experimental evidence to support this spatial hypothesis for the rapidly cycling subset? At hippocampal synapses, recently recycled vesicles do tend to be somewhat preferentially located near the active zone, although the degree of concentration is not large (Schikorski and Stevens, 2001). But at the neuromuscular junction, Rizzoli and Betz (2004) examined the structural basis for the rapidly cycling pool and found results inconsistent with the kiss-and-run scheme. They labeled the rapid pool with the styryl dye FM1-43, which associates with the lumen of vesicles during exocytosis and is then trapped by endocytosis. After photoconversion of FM1-43 to form an electron-dense product, endocytosis was observed by electron microscopy at sites distant from the active zone rather than at the release sites as expected for kiss-and-run. Furthermore, labeled vesicles mingled among the unlabeled (reserve) vesicles, indicating that something other than proximity to release sites confers rapid-release status on the recently recycled vesicles.

Evidence Favoring Kiss-and-Run

Although kiss-and-run does not seem to maintain the rapidly cycling pool at the neuromuscular junction, some studies suggest that kiss-and-run may occur at synapses of cultured hippocampal neurons. Gandhi and Stevens (2003) used virus-mediated overexpression of a fluorescent reporter, synaptopHluorin, to estimate the dwell time of single vesicles in the exocytic state. SynaptopHluorin is the vesicle membrane protein VAMP with pH-sensitive GFP attached to its luminal end, exposed to the vesicle interior where its fluorescence is normally quenched by the high intravesicular proton concentration used to support neurotransmitter uptake. During exocytosis, the protons escape and fluorescence increases until the vesicle pinches off and reacidifies. One complication is that overexpressed synaptopHluorin is present not only in vesicles but also on the plasma membrane, where its high fluorescence contributes a substantial background. Gandhi and Stevens were able to reduce this background by photobleaching, which allowed detection of remarkably constant quantal steps that they attributed to single-vesicle fusions during stimulation. Although photobleaching solved the background problem, it is not clear what other effects the photobleached SNARE protein in the plasma membrane may have and whether it might exchange with vesicle synaptopHluorin during exocytosis (VAMP from vesicles...
The Fate of Retrieved Membrane after Compensatory Endocytosis

The upside of kiss-and-run is that it neatly solves the problem of sorting vesicle components from the plasma membrane. The downside is that a crucial site at the active zone is occupied by a nonfunctional vesicle after an exocytic event. For a synapse that releases with low probability and is stimulated at low frequency, this may be an acceptable price. But the situation is apparently different at synapses that transmit with high probability, at high frequencies, or continuously. For example, ribbon synapses of the retina release neurotransmitter continuously for prolonged periods. In ribbon synapses of retinal bipolar cells, direct imaging of single vesicles labeled with FM1–43 reveals rapid mixing of vesicle and plasma membranes during exocytosis, consistent with full fusion rather than kiss–and–run (Zenisek et al., 2002). If not kiss–and–run, then what method of endocytosis is used at the bipolar cell synapse? Perhaps the best–known mechanism for endocytic retrieval is clathrin–dependent endocytosis, illustrated in pathway B of Figure 1. However, electron microscopy detected no coated vesicles and pits associated with synaptic activity in bipolar neurons, as would be expected for clathrin–dependent retrieval. Instead, compensatory endocytosis at the bipolar cell synapse involves infoldings of the plasma membrane that pinch off to form large endosomes (Paillart et al., 2003; Holt et al., 2004), which later give rise to new synaptic vesicles (pathway C, Figure 1). A similar mechanism of bulk retrieval also operates at ribbon synapses of hair cells (Lenzi et al., 2002), although in this case coated vesicles and pits were also observed, arising either from plasma–membrane invaginations or from internalized endosomes.
Photoreceptors also have ribbon-type synapses that release neurotransmitter continuously, but both photoreceptor terminals do not use bulk membrane retrieval like bipolar neurons and hair cells (Rea et al., 2004). Instead, after uptake and photoconversion of FM1–43, internalized label was confined to synaptic vesicles, which were generated via clathrin-dependent endocytosis without intermediate cisternae (pathway B, Figure 1). Thus, endocytosis mechanisms are diverse even when comparing synapses that have a similar structural organization. In bipolar cell terminals, only ~10% of vesicles were labeled after >10 min of repetitive activity, reflecting the relatively slow reformation of vesicles from large endosomes (Paillart et al., 2003). By contrast, ~80% of vesicles were labeled in photoreceptor terminals under similar conditions, demonstrating rather complete participation of all 250,000 vesicles in the photoreceptor vesicle cycle. What accounts for the difference? Both bipolar cell terminals and cone terminals contain several hundred thousand vesicles, but the photoreceptor terminal is able to sustain high output for prolonged periods in the dark, when the cell is depolarized. To do so, photoreceptors depend on a large, highly mobile pool of releasable vesicles that must be rapidly replenished without going through a slower endosomal intermediate. The bipolar cells studied by Paillart et al. depolarize in response to illumination but are less able to maintain prolonged release. Thus, the hundreds of thousands of vesicles in the bipolar-cell terminal are evidently able to keep up with demand without requiring a rapid resupply of recycled vesicles. In keeping with the relative demands for resupplying the active zones in photoreceptors and bipolar cells, vesicles in bipolar-cell terminals are about 10-fold less mobile than in photoreceptor terminals (cf. Rea et al., 2004; Holt et al., 2004).

The neuromuscular junction and the calyx of Held are conventional synapses that have multiple active zones and large numbers of vesicles, similar in this regard to ribbon synapses. Recycling at these synapses shares features with both photoreceptors and bipolar cells, depending on conditions. At both synapses, a rapidly cycling subset of vesicles is able to maintain neurotransmitter release by balancing release and recycling, but reserve vesicles are tapped if demand for release exceeds the capacity of the rapid pool. Recycling of reserve vesicles proceeds via membrane invaginations and intermediate endosomes (cisternae) in both cases (Richards et al., 2003; de Lange et al., 2003), whereas recycling of the rapid pool is more direct. Activity-dependent slowing of endocytosis has also been observed at the calyx of Held using measurements of membrane capacitance—an index of surface area—during endocytosis (Sun et al., 2002). This is reminiscent of a similar slowing of endocytosis with prolonged stimulation at bipolar cell synaptic terminals (von Gersdorff and Matthews, 1994), but the fastest release rate at the calyx (τ = 50–100 ms for single vesicle fusions; Sun et al., 2002) is more rapid than the fastest rate at bipolar cell terminals (τ = 1 s). The multiple kinetic components of endocytosis observed in capacitance measurements can be differentially affected by experimental manipulations (e.g., Heidelberger et al., 2002) and likely reflect the operation of different underlying pathways for membrane retrieval.

**Remaining Issues**

Although mechanisms for vesicle recycling are diverse, some general features also emerge, such as a switch from rapid to slower forms of recycling with increasing amounts of release (an exception is the photoreceptor synapse, which lacks a reserve pool and relies on rapid reformation of a large, mobile pool of releasable vesicles). How might the switch from fast to slow mode be regulated? Perhaps it isn’t. Conceivably, the machinery for bulk retrieval is always active but is simply outcompeted by more rapid mechanisms that have limited capacity. It is also commonly true that rapidly retrieved vesicles preferentially refill the readily releasable pool, whereas vesicles regenerated by slower bulk retrieval tend to end up in the reserve pool. How are vesicles retrieved by the fast mode tagged for preferential reuse in subsequent bouts of exocytosis? Without knowing what distinguishes at the molecular level the rapidly cycling vesicles from the reserve pool, it is difficult to answer this question, but proximity of rapidly retrieved vesicles to the active zone is evidently not the sole answer (e.g., Rizzoli and Betz, 2004). Presumably, different endocytic paths lead to distinct biochemical states or differential cytoskeletal attachment of vesicles, which in turn influences how readily the vesicle can be recycled. But a more satisfying answer must await information regarding the molecular basis of the clathrin-independent endocytosis mechanisms at the synapse, about which little is known. Because bulk retrieval seems to be involved in vesicle recycling at a variety of synapses, identification of the molecular mechanism(s) involved in this mode of recycling should be a priority.

The long-standing question of kiss-and-run exocytosis also remains an issue. At large synapses like the neuromuscular junction and retinal bipolar neurons, the evidence suggests that kiss-and-run is not a major player in the vesicle cycle. In the CNS, space constraints require small synaptic terminals with room for relatively few vesicles, a situation that could favor kiss-and-run or kiss-and-stay recycling. There is some disagreement about the degree to which kiss-and-run actually contributes to vesicle recycling at small CNS synapses, but if it does occur, the kiss-and-run mode may require conditions that also affect the probability of release per action potential. Gandhi and Stevens (2003) reported that kiss-and-run is dominant at hippocampal synapses with low release probability. If this relation turns out to be general, what might the connection be between release probability and the kiss-and-run mode? Reversibility of the fusion pore may require a different state of the pore or the local plasma membrane, either of which could in turn affect release probability. In this regard, recent suggestions of a link between the fusion pore and SNARE proteins are especially intriguing (Bai et al., 2004; Han et al., 2004). Furthermore, if the fusion pore is sufficiently small that molecules like HEPES and FM dyes are excluded, then neurotransmitter might also leak out slowly through the open pore. It is not clear what the postsynaptic response to such slow release would look like, especially at synapses where the postsynaptic receptors are tuned for fast transmission. Another implication of release through a fusion pore is that the amount of neurotransmitter release from a single vesicle could depend on pore conductance and open
duration, which might offer a new avenue for presynaptic regulation of synaptic efficacy (e.g., Barclay et al., 2004). Of course, regulation of the fusion pore becomes of little significance if it turns out that most neurotransmitter release proceeds by full fusion. But whatever the role of kiss-and-run turns out to be, the increasing availability of optical methods to track vesicle cycling in a wider variety of synaptic preparations should help establish which of several alternative paths for exo/endocytic cycling is preferred under a particular set of physiological circumstances.

Selected Reading