

THE SYNAPTIC VESICLE CYCLE

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Key words: fusion pore; exocytosis; endocytosis; trafficking; secretion

Shortened title: SYNAPTIC VESICLE CYCLE

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ABSTRACT

The ins and outs of the synaptic vesicle cycle are being examined in increasing detail with diverse investigative tools in a variety of cell types, particularly those with large granules. The cycle begins with the opening of a fusion pore that connects the vesicle lumen to the extracellular fluid. Sensitive electrophysiological techniques reveal the often-stuttering behavior of single pores in non-neuronal cells, through which small molecules trickle until the fusion pore expands and the remaining contents erupt from the vesicle. The granule membranes are then retrieved by multiple processes that appear to act in parallel, and can be distinguished from each other kinetically and ultrastructurally. Next, following endocytosis, synaptic vesicles are shuttled back into the vesicle pool, where they mix with other vesicles for a while, but then become immobilized, and remain gelled with their neighbors even while moving again to the presynaptic membrane as a prelude for another round of exocytosis.

The two major features of presynaptic function - the quantal theory and the vesicular hypothesis - are now more than forty years old. Proof of secretion via multimolecular quantal packets came early on [25]. The vesicular hypothesis, however, resisted definitive analysis; while abundant evidence consistent with the theory accumulated for several decades, it was not until the 1990's that the combined application of patch clamp capacitance and amperometry provided virtual proof that transmitter secretion is indeed coincident with exocytosis [2,13]. Now, the entire vesicle cycle is studied more intensively than ever, thanks largely to new tools of molecular analysis, electrophysiological monitoring, and optical imaging. Various aspects of the cycle, including the identification of molecules involved in docking and exocytosis and the role of calcium in the vesicle cycle, have been reviewed recently [4, 56,67]. Here we will focus on three different aspects of the cycle, namely the behavior and fate of the fusion pore, the nature of endocytosis, and the properties of

intracellular trafficking of recycling vesicles. Our understanding, particularly of the first two of these events in presynaptic terminals has been informed greatly by experiments on non-synaptic cells, particularly adrenal chromaffin and other neuroendocrine cells and mast and other leukocytic cells. The reasons for this are twofold: technically, the non-synaptic cells can be patch clamped and therefore studied with the capacitance technique; biologically, the non-synaptic cells secrete from granules that are significantly larger than small, clear synaptic vesicles, and thus give bigger capacitance and amperometric signals. The increasing application of these techniques to neurons is beginning to allow more direct comparisons. Capacitance monitoring has been performed with posterior pituitary nerve terminals [24,31,48], goldfish retinal bipolar cells [17,60,61], photoreceptors [46,47], and saccular hair cells [43], and amperometry has been applied to leech neurons [9] and mammalian superior cervical ganglion cells [27,66].

THE FUSION PORE

Flickers, feet, and spikes

Elegant and sensitive recording techniques have revealed beautiful details of the fitful and unpredictable lives of fusion pores. Electrical properties of individual fusion pores have been studied most thoroughly in cells that contain relatively large granules. Fusion pores open abruptly to conductances typically of several hundred picoSiemens [2,53]. Their subsequent fate is unpredictable. They may ‘flicker’ between closed and open states for a variable period that can last for several seconds, and then ‘explode’ open or close tightly [53]. Two examples of such behavior, monitored with different recording techniques, are illustrated in Figure 1. Four ‘foot-spike’ events recorded by amperometry [63] from a bovine adrenal chromaffin cell are shown in Figure 1A [65]. In this sensitive technique, a carbon fiber electrode, clamped at a potential of about +800 mV, is positioned close to the cell. Catecholamines released during single exocytic events strike the electrode and are oxidized or reduced, generating the amperometric signals. These traces illustrate how small amounts of catecholamines may dribble through a nascent fusion pore (the ‘foot’) for more than one-hundred milliseconds before the pore explodes open to release the remaining contents of the granule (the ‘spike’). During the foot, the signal may fluctuate, as if the fusion pore size was rapidly wavering. These ‘amperometric flickers’ are considerably faster than ‘capacitance flickers’ described above. In chromaffin cells, 50-70 per cent of all spikes are preceded by a foot that lasts about 50 ms and releases about 10% of the total contents of the granule. About one-third of all feet show distinct flickers; on average each foot flickers 2.7 times at a frequency of about 170 Hz before the spike. Elevation of $[Ca^{++}]_i$ increases the number and frequency of flickers [65].

Some feet are not followed by a spike. In these ‘foot-alone’ events, the average duration of the foot is longer by a factor of two (to about 100 ms) than in ‘foot-spike’ events. The evident explanation of foot-alone events is that the fusion pore opens, flickers awhile, and closes. Although other explanations, such as the controlled release from a granular matrix [44] cannot be ruled out with certainty at present, this is of course strong evidence for the phenomenon known colloquially as ‘kiss and run’ exocytosis.

Reversible openings of fusion pores can also be recorded with the whole cell capacitance technique. This technique tracks the increase in plasma membrane surface area that accompanies exocytosis, and decrease in area that reflects endocytosis [40]. Whole cell capacitance lacks the sensitivity of amperometry, and thus for detection of single exocytic and endocytic events cells with large granules, such as mast cells, are typically studied. The top two traces of Figure 1B were recorded from a normal mast cell as it underwent exocytosis. Each upward step reflects the exocytosis of a single granule. In three cases (marked *) the events were transient, and reversed abruptly after several hundred milliseconds. The lower pair of traces in Figure 1B were recorded from a mast cell obtained from a mutant (‘ruby-eye’) mouse [42]. The traces are far more complex than those from the wild type

(upper pair of traces), showing many more examples of transient events. The molecular basis of this mutant behavior is not known.

Combined amperometry and capacitance of ruby-eye mouse mast cells has revealed further interesting behavior: during the prolonged exocytic burst evoked by a stimulant, the initial amperometric spikes are relatively small. Over time (a few minutes), the amperometric spikes become larger, even though the capacitance jumps remain unchanged. These results suggest that all granules are the same size (no change in capacitance step size over time), but that the first ones to undergo exocytosis contain less serotonin (the normal transmitter) than do the later ones. Such a situation could have arisen if the originally docked granules had become partially depleted of stores owing to spontaneous transient openings of the fusion pore before the exocytic stimulus was applied - a sort of granular incontinence. This hypothesis was confirmed by preincubating cells in serotonin, which restored the initial amperometric events to normal amplitude, presumably by preventing leakage during the spontaneous openings that preceded the experimental recordings [42].

Do studies like these provide definitive proof of 'kiss and run' exocytosis? In each individual case, it is possible to imagine alternative explanations. The real force of the evidence comes from complementary results obtained using different techniques. In addition to amperometry and whole cell capacitance, other approaches offer promise. For example, exocytosis of single granules (about 1 μm in diameter) in sea urchin eggs can be visualized directly with the light microscope [62]. These granules secrete large amounts of protein, and thus their fusion pores must dilate. Fluorescent dye labeling showed that endosomes that form after exocytosis are the same size as the secretory granules. Moreover, serial application of different colors of dyes did not give endosomes with mixed colors, suggesting that they (the endosomes) did not form by coalescence of smaller vesicles, but pinched off whole [62]. These observations of course suggest that endosomes may form directly as dilated fusion pores shrink and close. This preparation affords an opportunity to test directly this hypothesis, by observing single stained granules over time while monitoring (perhaps with dyes of different colors) the fusion pore patency.

Other new techniques offer additional exciting possibilities. 'Patch amperometry' [1] provides a significant increase in capacitance sensitivity owing to the cell-attached recording configuration [34,40], and with the carbon fiber placed inside the patch pipette, every exocytic event that occurs in the patch is detected amperometrically. 'Evanescent wave microscopy' offers a means to visualize single stained granules that lie close to (within about 300 nm of) of the plasma membrane [54]. Serotonin-containing granules can be visualized in unstained cells by using 'multi-photon' microscopy [35]. Luminescent fusion proteins ('synaptolucins') inside synaptic vesicles light up after exocytosis [37].

Fat or meat?

The molecular structure of the fusion pore is not known with certainty. It is natural to consider its behavior like that of an ion channel, or a gap junction, proteins that are initially gated open to a conductance of up to several hundred pSiemens (like a fusion pore), and may transiently flicker and close [32]. The normal full dilation of the fusion pore would require that the protein subunits burst apart when the fusion pore explodes open and the lipids of the granule and plasma membranes coalesce. Some evidence is consistent with this idea [32]. For example, the behavior of fusion pores of single, 100 nm diameter granules in human neutrophils has been studied using the cell-attached recording mode, which increases by more than ten-fold the sensitivity of capacitance recordings compared to whole cell mode [34]. In about one-half of the exocytic events, a discrete value of fusion pore conductance can be resolved (the pore opens abruptly to this level, then hovers for a few hundred milliseconds before dilating). It's average (about 150 pS, which was independent of the capacitance step (i.e., granule size)) is like that of an ion channel [34], although the recorded range was large (coefficient of variation = about 0.6) compared to most ion channel recordings. Considerable variability in fusion pore conductance has also been observed in mast cells [53].

Alternatively, one can envision that the initial fusion pore is lined not with proteins, but with lipids, and that the proteins play other roles in catalyzing and regulating interactions between the lipids of the granule and the lipids of the plasma membrane [39,12]. Evidence in support of this view includes the observation that lipidic pores can evidently shut and open. For example, capacitance recordings sometimes reveal transient openings that close to a *lower* capacitance value than that preceding the opening, as if membrane had flowed from the surface into the granule while the fusion pore was open [38]. The rate of lipid flux can be high - enough to replace the fusion pore lipids every millisecond. Similarly, fusion pores with conductances much greater than any known ion channel (up to several nS) can flicker [53]. If such lipid-containing pores can close, and then reopen, it seems reasonable to consider this a plausible, or even likely mode of operation during the first opening event.

Hemifusion

While it is clear that both protein and lipid components of membranes play a role in the final steps of secretory membrane fusion, the contribution of each type of component to the structure of the fusion pore is still uncertain. Vesicle fusion in protein free systems using synthetic phospholipid vesicles and bilayers demonstrated the formation of hemifusion structures in which the lipids in the outer leaflet of the vesicle bilayer mixed with the lipids in the target bilayer before complete fusion of the two bilayers was observed [11]. Hemifusion structures play a central role in several lipidic models of formation of fusion pores [12,32,39]. The ability of hemifusion and complete fusion to occur in the in vitro system could be modified by addition of different lipids [11]. Interestingly the same lipids have been found to have similar effects on several biological fusion events [12]. Future reconstitution experiments with well defined components should prove useful in dissecting the contribution of various proteins and lipids to exocytotic fusion events.

Granules and vesicles

The studies of single granule exocytosis are certainly of interest in their own right, and the ability to extend them to single synaptic vesicles would be of even greater interest to neurobiologists. The instrumentation sensitivity necessary to record the exocytosis of a single 60 nm diameter synaptic vesicle (about 0.1 fF capacitance change) has recently been approached with cell-attached capacitance monitoring [34]. At present, however, one can only speculate about the fate of a synaptic vesicle after exocytosis, drawing on knowledge of secretion from larger granules. That many of the molecules identified as participants in docking and exocytosis are contained in both cell types suggests close parallels in secretory mechanisms. Moreover, theoretical calculations show that a brief (1 ms) opening of a small (1 nm diameter) fusion pore would be sufficient to cause the release of virtually all (~99%) of the acetylcholine contained in a synaptic vesicle [53,55], which could then refill with the transmitter in several seconds and be ready again to participate fully in an exocytic event.

Despite these parallels, and despite the intuitive appeal of such a conceptually simple mechanism, there exists virtually no quantitative, direct evidence to support the notion that small synaptic vesicles ever flicker transiently open. On the contrary, electron microscopic evidence gives strong evidence that synaptic vesicles can and do collapse into the plasma membrane and many seconds later are reinternalized at relatively remote sites (about 1 μ m from the exocytic sites) [22]. Moreover, measurements of the rates of uptake of FM1-43 after stimulation offer virtually no evidence of a rapid (subsecond) process in hippocampal [51] and frog motor [64] nerve terminals. Several observations are, however, difficult to explain at present. For example, frog motor nerve terminals, fixed immediately after intense stimulation, fail to stain extensively with antibodies to synaptophysin (a vesicle membrane protein). The intense stimulation should have shifted lots of vesicle membrane to the surface if endocytosis is slow; the lack of staining suggests that the synaptophysin-containing membrane was reinternalized relatively quickly [59]. In addition, some vesicular structures stained with FM1-43 or other styryl dyes do not destain as readily as expected under some conditions (e.g., frog motor nerve terminals treated with staurosporine [18], hippocampal cultures treated with

ruthenium red (P.Haydon, personal communication), mammalian primary afferent terminals (M. Chua & C.C. Hunt, personal communication)).

This situation produces something of a paradox: compelling evidence for rapid ‘kiss and run’ exocytosis exists where it is not particularly effective in secreting the contents of the exocytosing granule (e.g., amperometric ‘feet’ release only a few per cent of the total contents), and virtually no solid evidence for ‘kiss and run’ exocytosis exists where it would be entirely sufficient to rid a vesicle of all of its transmitter molecules (e.g., a small, clear synaptic vesicle). The paradox is at least partly resolved by noting that the secretion of small amounts of a granule’s contents may be physiologically important - perhaps partial secretion is useful in permitting a finer level of control than all-or-nothing exocytosis. Moreover, the slower, more complex and laborious route of recycling evidently followed by synaptic terminals would not be the first example of Nature eschewing apparent parsimony. Finally, it is sometimes assumed that dense core granules are in the business of secreting their dense cores, which requires fusion pore dilation. This is undeniably true for cells whose main secretory product is protein, like pancreatic beta cells secreting insulin. More recently, evidence has emerged that granules containing small secretory molecules, like catecholamines in adrenal chromaffin cells and serotonin in mast cells, contain a matrix that is probably not destined for secretion. Instead, the matrix comprises at least some molecules that act as an ion exchange resin that functions to maximize the amount of small transmitter molecules contained in the granule [44] - a kind of serotonin sponge in mast cell granules, for example. Recycling, rather than secreting such a commodity could clearly be beneficial during granule recycling. If fusion pore dilation increases the risk of losing of such a matrix, then the utility of ‘kiss and run’ exocytosis is evident.

ENDOCYTOSIS

Secretory granules show clear evidence that they possess fusion pore assemblies that can, on occasion, open briefly and then close - ‘kiss and run’ exocytosis in its purest form. Most of the time, however, the fusion pore explodes open and dilates. The subsequent fate of the granule is nearly as big a mystery as that of a post-exocytic synaptic vesicle. The range of possible destinies after a fusion pore dilates is abundant; issues of both ‘essence’ and ‘substance’ of a vesicle are involved. For example, a vesicle might retain its ultrastructural appearance after exocytosis (‘essence’ intact) while the flux of lipids and other membrane constituents through the fusion pore changes entirely its molecular composition (‘substance’ altered). At the opposite extreme, the vesicle might collapse and disappear, and stream as unit to a remote site of endocytosis, its essence thereby vanishing, while its substance remains unaltered. Countless plausible intermediate routes also exist, and it appears increasingly likely that at least two different, parallel routes of endocytosis exist.

Capacitance studies in several cell types, including nerve terminals of the goldfish retinal bipolar cell [60,61], have consistently revealed a relatively rapid endocytic process (time constant of a few seconds) that is tightly coupled to preceding exocytosis. Examples are shown in Figure 2A. It is of course tempting to suppose that the rapidly internalized membrane is the same as that which underwent exocytosis, which would almost certainly require that it be mediated by what is conventionally envisioned as ‘kiss and run’ exocytosis (clathrin-mediated endocytosis would be slower). However, it is also possible that the captured membrane is entirely different than the exocytic vesicle membrane, and that the two events just happen to be tightly coupled in time. In fact, evidence for such a linkage exists: under some conditions a phenomenon called ‘primed endocytosis’ is observed in capacitance studies of adrenal chromaffin cells. This takes the form of an extremely fast, short latency endocytic event (‘primed’ endocytosis), so fast that it masks, or even reverses the stimulus-evoked exocytic capacitance jump [41,52,58]. Primed endocytosis probably results from exocytic events that occur long before the stimulus is delivered; these exocytosed granules move slowly along a morphologically and functionally obscure pathway that leads ultimately to endocytosis, and accumulate at the last station along the route, poised for quick pinch-off. The applied stimulus then provides the trigger for that last,

internalizing step. It is possible that rapid endocytosis is a slower version of primed endocytosis, and that in both cases the membrane being retrieved is not the same as that which was exocytosed.

Most of the time endocytosis proceeds in steps that are too small to be resolved individually with whole cell capacitance recording, although in several studies individual abrupt drops in capacitance have been resolved clearly [34,40,48]. The final rate of closure of such ‘fission pores’ is slow compared to fusion pore opening [48]. The circumference decreases at a final rate of about 25 nm/s, which corresponds to the removal of about 10% of the pore-lining lipids each 15-20 ms [48]. In addition to fission rates, the amplitudes of endocytic steps have been measured, and the observed values vary widely in different capacitance studies. For example, in pituitary nerve terminals studied in the whole cell capacitance mode, single exocytic events were not resolvable, while some single endocytic events were large enough to be detected (most endocytic events, however, were also too small to be resolved individually) [48]. Thus, at least some endocytic events involved the internalization of amounts of membrane greater than that contained in secretory granules. Such events, which also have been observed in chromaffin cells [40] are most commonly seen after intense, perhaps non-physiological stimulation of exocytosis. Human neutrophils examined with the high-resolution cell-attached capacitance technique gave a different result [34]. Most increases and decreases in capacitance were resolvable as discrete steps (although not the same granules; endocytic steps were especially prevalent during the initial stages of recording, exocytic steps later, after the addition of ionomycin). The average endocytic step was only about half as large as the average exocytic step, suggesting that granules were perhaps retrieved piecemeal after exocytosis and fusion pore dilation - a fragmented ‘run’ following a protracted ‘kiss’.

Other synaptic terminals that secrete *via* small clear vesicles have been examined optically (Figure 2B). Two recent studies using the uptake of FM1-43 applied after tetanic nerve stimulation have shown that the endocytic process in nerve terminals is relatively slow; the fastest time constants observed were about 20 s [51,64], approximately ten times slower than ‘fast endocytosis’ observed with capacitance recordings in goldfish bipolar nerve terminals [60,61], pituitary nerve terminals [24,58], and neuroendocrine cells [3,21,41,58].

The *Drosophila* temperature-sensitive mutant *shibire* has been invaluable for investigations of endocytosis [28]. Its mutant protein, dynamin, mediates a final step in endocytosis, the pinching off of the endosome. In fact, none of the many proteins identified as players in the synaptic vesicle cycle is better understood than dynamin [15]. At elevated temperature, endocytosis is completely arrested, suggesting that dynamin is essential for any and all membrane internalization [28,45]. The situation, however, evidently does not involve a single pathway, for clear evidence of two parallel pathways of membrane internalization has been described [26] (Figure 3). One pathway is relatively slow, occurs at sites remote from the exocytic sites (active zones), and involves the generation of plump, branched tubules from which coated vesicles bud; this pathway evidently corresponds to the clathrin-mediated route originally described by Heuser and Reese. The second pathway is relatively fast and arises at sites adjacent to active zones as unbranched, flat cisternae that extend into the terminal cytoplasm. The two morphologically distinct routes naturally raise the possibility that two distinct types of recycled vesicles are generated [26]. While some evidence is consistent with this idea, the optical and ultrastructural observations made with FM1-43 do not suggest the presence of more than a single functional type of vesicle in significant numbers [5,20].

The great value of *Shibire* for studying the mechanisms of endocytosis lies in its selectivity and reversibility. Recently, a technique - intracellular acidification - has been shown to reduce endocytosis reversibly and selectively in lizard motor nerve terminals [33]. This clearly could become a valuable investigative tool, although the lack of effects of intracellular acidification on rapid endocytosis in adrenal chromaffin cells [3,10] and pituitary nerve terminals [58] shows that it is not applicable to all cell types.

TRAFFICKING

The trafficking of endosomes and the mechanisms that guide the regeneration of synaptic vesicles is the least understood aspect of the vesicle cycle [8]. One of the main reasons for our relative ignorance concerns a lack of investigative tools to study the process. In addition, cells that secrete *via* dense core granules may be less informative on this aspect of the cycle, since the trafficking route of recycling granules may be vastly different than synaptic vesicles. This is especially so if granules lose their protein matrix during secretion, for recycling would then require that they acquire new matrix proteins *via* communication with the golgi apparatus. A recycling synaptic vesicle can be separated from its golgi apparatus by a vast distance, and can be refilled easily with small neurotransmitter molecules that are synthesized locally in the cytoplasm of the nerve terminal.

The trafficking route of recycling vesicles can be divided into three phases: the endocytic phase, which begins with the pinching off of an endocytic vesicle, the steady state phase, which describes the situation for mature vesicles in a resting nerve terminal, and the mobilization phase, by which nerve activity moves a vesicle to the presynaptic membrane as a prelude to docking and exocytosis. Each of these can be profitably studied with imaging techniques; according to studies using FM1-43, it takes about a minute for a vesicle to pass through the complete cycle from exocytosis to exocytosis [5,6,8,49].

The endocytic phase. In non-neural cells, the components of early endosomes are sorted into different physical pathways by a series of selective vesicular fusions and fissions [36]. By analogy, it was thought that recycling synaptic vesicles may follow a similar course, although more recent data have suggested that no intracellular fusions are involved, that as soon as a clathrin-coated recycling synaptic vesicle is uncoated, it is ready to reenter the vesicle pool [57].

Three different studies have examined the question of whether recently recycled vesicles are sequestered, for example at the front or back of the cluster of 'reserve' vesicles, or not. All three studies have reached the same conclusion: recycled vesicles mix randomly within the pool of existing vesicles [5,29,49].

The resting phase. The observed mixing of recycling vesicles during the early stages of recycling naturally suggests a picture of 'fish in a bowl' - vesicles moving, if not churning, constrained to a cluster perhaps by some sort of cytoskeletal cage. On the other hand, considerable evidence has accumulated for more than a decade suggesting that synaptic vesicle motion in resting terminals is constrained. Electron microscopy shows clear signs of filamentous cross-links, which are likely to include the protein synapsin I, between vesicles [23,30]. Recently vesicle mobility in living, resting nerve terminals has been investigated in two studies. Each involved the Fluorescence Recovery After Photobleaching (FRAP) technique, and each gave the same result: little or no recovery from photobleaching occurred in resting terminals, suggesting that the vesicles are not mobile [19,29].

The mobilization phase. During repetitive nerve stimulation, vesicles move to the presynaptic membrane as a prelude to docking and exocytosis. What frees them from their resting bonds, and how do they get to the membrane? Concerning mobilization, *in vitro* studies have shown that phosphorylation of synapsin I by calcium-calmodulin kinase II reduces its (synapsin I's) affinity for synaptic vesicles [14]. Perhaps, then, synapsin I phosphorylation is the normal *in vivo* mechanism for freeing vesicles from their resting bonds. The next process - how the vesicles get to the membrane - is more of a mystery. A single FRAP study has dealt with this issue. Vesicle clusters, stained with FM1-43, were viewed from above and small spots were bleached with a laser. Then the nerve was stimulated and the terminals destained. The bleached spots did not recover during the destaining, suggesting that as vesicles moved to the membrane, their lateral movements were sharply constrained [19].

The simplest explanation of this result is that synaptic vesicles are glued to their neighbors (perhaps by synapsin I), and that they are passively pulled towards the presynaptic membrane as

docked vesicles exocytose and collapse into the surface membrane. In considering more complex mechanisms, one's attention is naturally drawn to the cytoskeleton, particularly actin (microtubules are excluded from synaptic vesicle clusters [23,30]). Synapsin I interacts with actin *in vitro* [14], raising the possibility that similar reactions - perhaps involving a molecular motor tugging a vesicle along an actin track - occur *in vivo* (although recycling rate is not affected in Synapsin I knockout mice [50]). A key unanswered question concerns the morphological disposition of actin filaments in nerve terminals in general, and in vesicle clusters in particular. Unfortunately, the presence, much less arrangement of actin filaments in vesicle clusters is not clear. Ultrastructural studies are not in complete agreement [23,30]. Light microscopic studies of actin in motor nerve terminals (where vesicle clusters are largest) are difficult to perform owing to the large background signal from muscle actin. A hopeful development in this regard is the work of Connor and colleagues [16], who abolished background muscle fluorescence by killing muscle fibers without damaging nerve terminals. Their observations suggest that actin is not codistributed with synaptic vesicle clusters, and do not rule out the possibility that actin may be excluded from vesicle clusters.

Clear evidence of 'active' movements of synaptic vesicles have been documented. Okadaic acid, a protein phosphatase inhibitor, disrupts synaptic vesicle clusters in resting terminals, evidently by unmasking powerful and widespread vesicle translocators. In frog motor nerve terminals stained with FM1-43 vesicles can be seen to stream from clusters in both directions, moving at rates of a few micrometers per minute (~50 nm/s) [7]. Do these motors normally transport vesicles to the presynaptic membrane? The answer is not known. Nerve stimulation does not ordinarily disrupt vesicle clusters the way that okadaic acid does, suggesting that if these motors are involved, they must be activated selectively by nerve stimulation.

CONCLUSION

Progress towards the holy grail (for neurobiologists) of capacitance studies - the detection of a single synaptic vesicular event - has been steady and although the techniques are arduous to implement and challenging to refine, the pace of progress seems to be accelerating as success draws nearer. Studies of endocytosis have revealed further information about the multiple, parallel cellular pathways by which membrane is internalized following exocytosis. For both exocytosis and endocytosis, our understanding of synaptic events has been greatly informed by studies of non-neuronal cells. As further information is obtained for different secretory cells and neurons, we will be able to determine the level of conservation and variation between different biological systems. Finally, studies of intracellular trafficking of recycling synaptic vesicles have barely begun to identify and characterize the mechanisms that regulate the movement of vesicles, a field that seems poised for further advancements.

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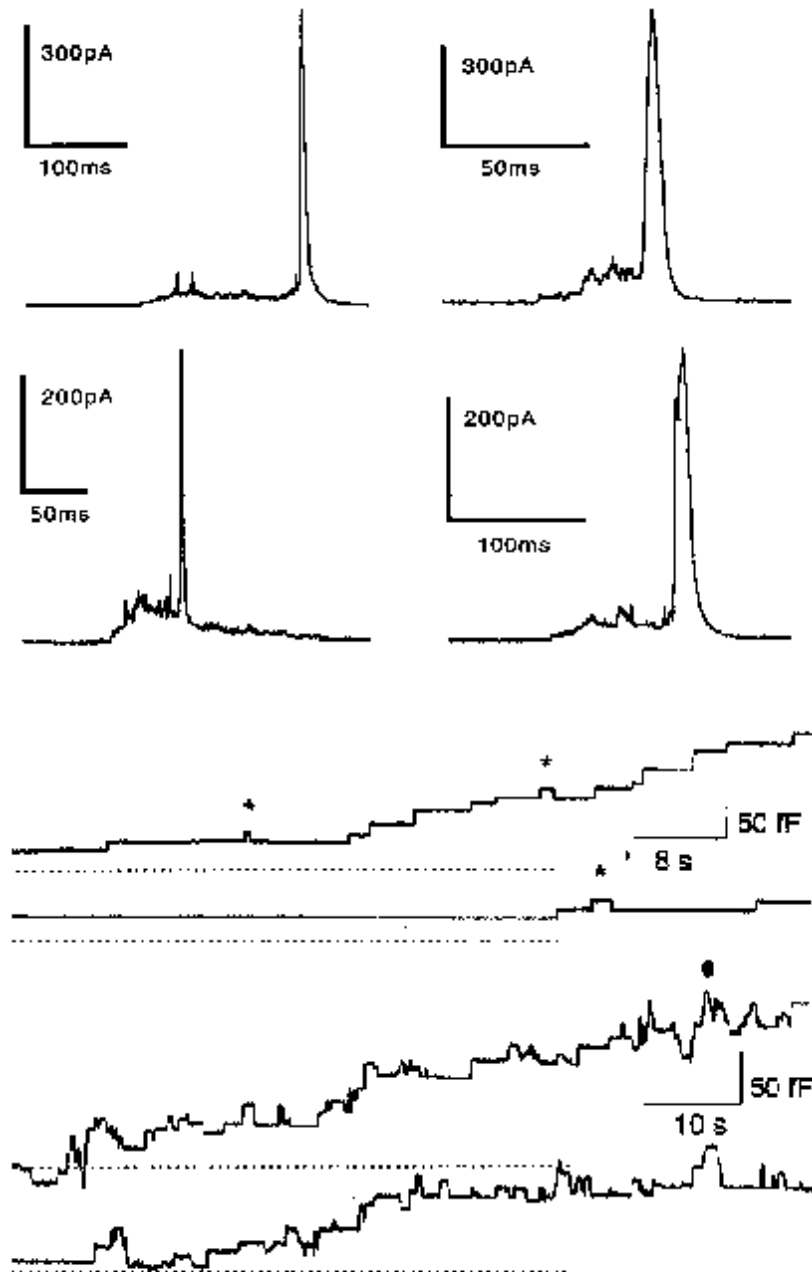


Figure 1. Fusion pore kisses and flickers. A. Four examples of 'foot-spike' events recorded by amperometry from an adrenal chromaffin cell [65]. The signal shows the current produced when secreted catecholamines strike a carbon electrode positioned near the cell. In each case, a low level of signal ('foot'), probably representing leakage of catecholamines through a small flickering fusion pore, precedes a 'spike' that probably results from the rapid dilation of the fusion pore. B. Whole cell capacitance recordings from a normal mast cell (top pair of traces) and from a 'ruby-eye' mutant mast cell (lower pair of traces) [42]. Each step reflects the exocytosis of a single secretory granule. Only three steps (marked *) are transient (i.e., reverse promptly) in the normal cell, while the mutant cell shows many more transients.

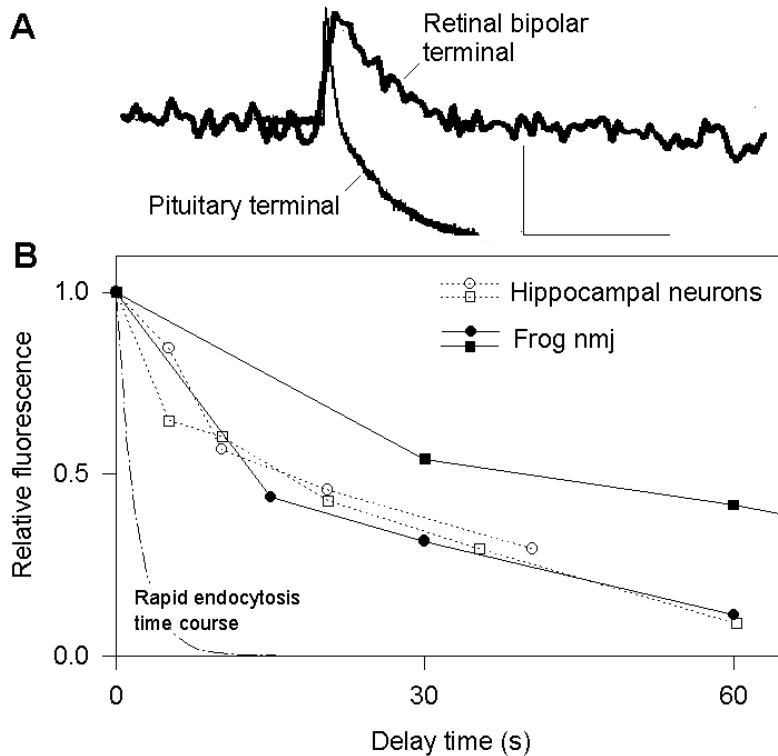


Figure 2. ‘Fast endocytosis’ seen with capacitance recordings is not observed with FM1-43 measurements of endocytosis kinetics. A. Whole cell capacitance recordings obtained from pituitary [58] and goldfish retinal bipolar [60] cell terminals. A short stimulus (not shown) evoked the abrupt increases in capacitance, which were followed by rapid decreases, probably reflecting endocytosis. Scale bars = 10 s and 100 fF. B. Endocytosis measured by the uptake of FM1-43 was slower in hippocampal neurons [51] and frog motor nerve terminals [64]. Each point was obtained by stimulating a preparation for a certain period of time, and then, after a ‘Delay time’ that ranged from zero to sixty seconds in different experiments, FM1-43 was applied and taken up by any remaining endocytic activity. The brightness of the terminals was then measured. The decay in brightness with increasing delay time provides a measure of endocytic rate. The shortest time constant was about 20 s, about ten times longer than rapid endocytosis shown in Panel A (dashed line).

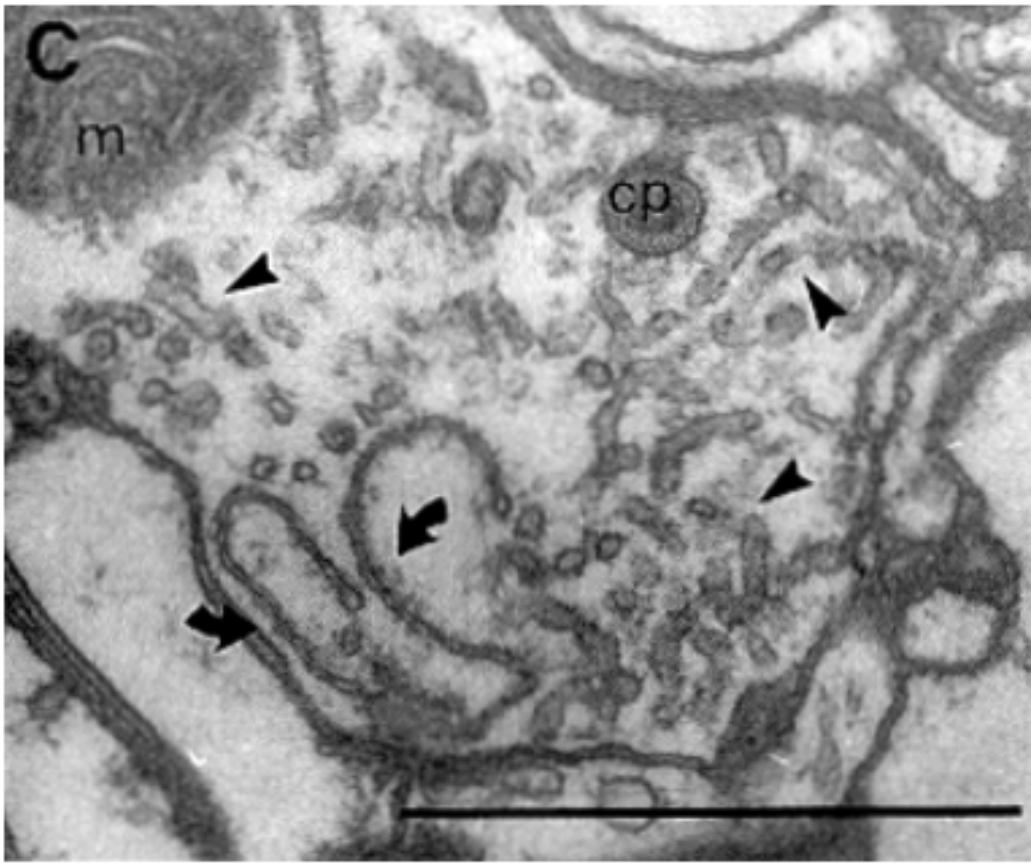


Figure 3. Two parallel routes of synaptic vesicle recycling in *Drosophila* photoreceptor cell [26]. Terminals were depleted of synaptic vesicles by stimulating with light at 29° for 30s, and then allowed to recover for 5 min at 26° C. *Arrowheads* point to branching, tubule-like endocytic structures that arise slowly at locations remote from exocytic sites. *Arrows* point to long, unbranching cisternae that emerge quickly at locations adjacent to exocytic sites (the dense body between cisternae is an exocytic site). Scale bar = 1 μm. m=mitochondrion; cp=capitate projection.