rière, O., Henderson, C.E., Haase, G., and Pettmann, B. (2002). Neuron *35*, this issue, 1067–1083.

Yi, F.H., Lautrette, C., Vermot-Desroches, C., Bordessoule, D., Couratier, P., Wijdenes, J., Preud'homme, J.L., and Jauberteau, M.O. (2000). J. Neuroimmunol. *109*, 211–220.

Yuan, J., and Yankner, B.A. (2000). Nature *407*, 802–809.

Zhu, S., Stavrovskaya, I.G., Drozda, M., Kim, B.Y., Ona, V., Li, M., Sarang, S., Liu, A.S., Hartley, D.M., Wu, du C., et al. (2002). Nature *417*, 74–78.

Synaptic Vesicle Exocytosis: Does a Lingering Kiss Lead to Fusion?

Direct optical measurements of single synaptic vesicles undergoing exocytosis at a synapse reveal rapid and complete transfer of membrane marker from the vesicle to the plasma membrane (Zenisek et al., 2002; this issue of *Neuron*). Contact between the two membranes is consistent with free lipid exchange, such as might result from full fusion of the vesicle and plasma membranes.

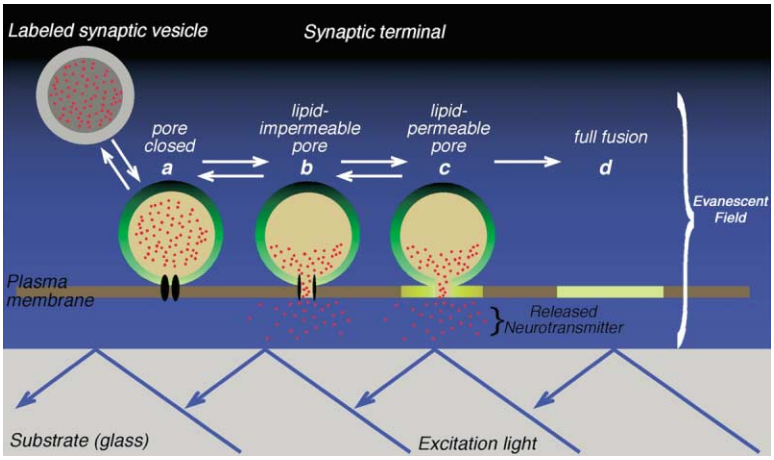
Does a synaptic vesicle release its neurotransmitter content at the synapse by fully fusing with the plasma membrane, followed by subsequent retrieval of the added membrane via a separate endocytosis step? Or, does the vesicle kiss and run, releasing neurotransmitter through a fusion pore that transiently connects the interior of the vesicle to the extracellular space? The debate over these two fundamentally different views of the exocytosis/endocytosis cycle at the synapse has continued for some 30 years. Lately, the kiss-and-run view seems to be gaining ground (e.g., Klingauf et al., 1998; Alés et al., 1999; Stevens and Williams, 2000; Verstreken et al., 2002), but a variety of evidence also favors full fusion. It is likely that both can occur, but it is not yet clear which is dominant and under what conditions.

The durability of the debate reflects in part the difficulty of obtaining direct information about vesicle fusion during neurotransmitter release because of the small size of both synaptic vesicles and synaptic terminals. Consequently, studies of vesicle exocytosis at synapses (as distinct from other secretory cells where more direct measurements are feasible) have commonly relied on indirect measures, such as the rate and degree of macroscopic destaining of whole terminals labeled with dyes that leave the vesicle membrane at different rates during exocytosis (Klingauf et al., 1998; Stevens and Williams, 2000). With recent technical advances, this situation is changing. For example, in this issue of *Neuron*, Zenisek et al. (2002) have directly viewed the destaining of individual synaptic vesicles at a glutamatergic synaptic terminal. Their work shows that marker dye incorporated into the vesicle membrane mixes with the plasma membrane within milliseconds when exocytosis is triggered by opening calcium channels. This rapid commingling of lipid demonstrates that the fusion pore does not present a significant barrier, on a millisecond time scale, to lipid exchange between the two membranes during neurotransmitter release. Further, single vesicles typically destained completely, which indicates that the vesicle kiss lingers for a while, possibly sufficiently long for the kiss to evolve into full-blown fusion of the vesicle and plasma membranes.

How did Zenisek et al. make these measurements? To observe single vesicle fusions, they used evanescent field microscopy (see Figure), a technique in which fluorophores within 100 nm or so of the substrate are excited by an excitation beam that is totally reflected at the interface between the substrate and the overlying cell. Another significant factor was the choice of giant synaptic terminals of retinal bipolar neurons as the experimental preparation. These large, glutamatergic terminals allow direct voltage-clamp control of the presynaptic terminal (Heidelberger and Matthews, 1992), which is important for accurate timing of the trigger for exocytosis. After a small subset of synaptic vesicles was labeled with the lipophilic dye FM 1-43, resident vesicles at the plasma membrane were triggered to fuse by activating calcium channels with brief depolarizations. Fusion was indicated by sudden brightening of the dye, followed by slower lateral diffusion of the dye in the plane of the membrane, away from the site of fusion (see Figure). The brightening occurred for two reasons. First, the dye moves closer to the substrate, and so to a more intense portion of the evanescent field, as it diffuses from the vesicle membrane into the plasma membrane. Second, as detailed in Zenisek et al. (2002), the dye molecules in the planar plasma membrane are more uniformly oriented with respect to the polarization of the excitation light than the dye molecules in the spherical vesicle membrane.

In one variant of the kiss-and-run notion, the fusion pore is presumed to be formed of proteins, conceptually similar to gap junction channels, that span the vesicle and plasma membranes and establish an aqueous pore through which small molecules can exit the vesicle into the synaptic cleft during the kiss phase (see Figure, [B]). The putative protein pore hinders lipid exchange between the vesicle and plasma membranes so that the marker dye in the vesicle membrane can escape only by desorbing from the vesicle membrane—a slow process—and exiting through the pore (e.g., Klingauf et al., 1998). By contrast, Zenisek et al. observed that dye left the vesicle and entered the plasma membrane on a time scale of milliseconds, which requires a direct diffusional connection between the two membranes (see Figure, [C]). However, Zenisek et al. also reported an appreciable delay, amounting to about 10 ms, between a brief depolarization and the release of dye to the plasma membrane.

What transpires during the delay between the onset of the depolarizing stimulus and the first brightening indicative of lipid diffusion? Part of the delay may represent the time required for calcium-dependent steps to trigger exocytosis (Heidelberger et al., 1994), although this is likely to account for only a millisecond or two, at most. Delays from the onset of depolarization to the opening of calcium channels may add another millisecond or so, leaving several milliseconds still unaccounted for. The video imaging system used by Zenisek et al.



Schematic Diagram of Hypothetical Steps in Synaptic Vesicle Exocytosis Viewed with Evanescent Field Microscopy

Excitation light is reflected at the internal surface of the glass substrate, setting up an evanescent field (blue gradient) that declines exponentially with distance from the site of reflection. A synaptic vesicle labeled with FM 1-43 fluoresces within the evanescent field at an intensity that depends on its distance from the plasma membrane. The steps are shown as a linear, reversible sequence, but it is not clear that all steps actually occur or that they must occur in sequence (see text). Full fusion (D) is shown as irreversible because retrieval of the fused membrane is thought to take place via independent mechanisms, such as clathrin-dependent endocytosis.

unfortunately did not have sufficient temporal resolution to decide whether this remaining delay represents a transient dwell time in a lipid-impermeable kiss (see Figure, [B]), followed by explosive dye release or direct transition to a lipid-permeable pore that then allowed lipid release with a time constant of a few milliseconds (see Figure, [C]). Nevertheless, a delay of a few milliseconds is sufficiently long for substantial neurotransmitter expulsion, even through a pore with the small conductance reported for synaptic-like microvesicles in pituitary nerve terminals (~19 pS; Klyachko and Jackson, 2002).

The question remains, however, how long the kiss lasts before running begins, if it begins at all. The apparently complete destaining observed by Zenisek et al. suggests that kissing must persist long enough after the pore becomes lipid-permeable to allow FM 1-43 to completely leave the vesicle membrane. So, the kiss is a lingering kiss. After this time, which may last for tens of milliseconds, the lipid-permeable fusion pore could close and disassemble in a process invisible to Zenisek and colleagues because the vesicle has now lost its marker. Thus, their results do not rule out the possibility that the vesicle somehow retains its structure in the face of lipid exchange and then breaks contact with the plasma membrane (see reverse arrows in Figure). However, a simple interpretation of the results is that lipid permeation during the kiss actually represents full incorporation of the vesicle membrane, which may be promoted by factors such as the relative membrane tension of the vesicle and the plasma membrane once the two membranes are in continuity.

In any event, Zenisek et al. interpreted their results as indicating that kiss-and-run is rare at the bipolar cell synapse. A similar conclusion was reached by Klyachko and Jackson (2002), who recorded capacitance jumps corresponding to the fusion of small-diameter, synaptic-like microvesicles in pituitary nerve terminals. In the latter experiments, capacitance flickers representing kiss-and-run exocytosis were rare (<5% of spontaneous events), and the initial fusion-pore conductance could not be resolved during irreversible exocytosis of single microvesicles, which suggests a rapid pore expansion to a high conductance. Such pore expansion is consistent with full fusion. Although the direct measurements of single-vesicle exocytosis reported by Zenisek et al. (2002) and Klyachko and Jackson (2002) push the pendulum back toward the full-fusion idea for vesicle exocytosis, there is still plenty of wriggle room. So, we have not yet heard the final word in the longstanding debate over the physiological significance of kiss-and-run exocytosis at synapses. And that's a safe prediction.

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Selected Reading

Alés, E., Tabares, L., Poyato, J.M., Valero, V., Lindau, M., and Alvarez de Toledo, G. (1999). Nat. Cell Biol. *1*, 40–44.

Heidelberger, R., and Matthews, G. (1992). J. Physiol. (Lond.) *207*, 623–633.

Heidelberger, R., Heinemann, C., Neher, E., and Matthews, G. (1994). Nature *371*, 513–515.

Klingauf, J., Kavalali, E.T., and Tsien, R.W. (1998). Nature *394*, 581–585.

Klyachko, V.A., and Jackson, M.B. (2002). Nature *418*, 89–92.

Stevens, C.F., and Williams, J.H. (2000). Proc. Natl. Acad. Sci. USA *97*, 12828–12833.

Verstreken, P., Kjaerulff, O., Lloyd, T.E., Atkinson, R., Zhou, Y., Meinertzhagen, I.A., and Bellen, H.J. (2002). Cell *109*, 101–112.

Zenisek, D., Steyer, J.A., Feldman, M.E., and Almers, W. (2002). Neuron *35*, this issue, 1085–1097.