Synaptic vesicles really do kiss and run

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A new study demonstrates that small synaptic vesicles exocytose dopamine through a flickering fusion pore almost exclusively, a process known as 'kiss-and-run' exocytosis. This process is driven by the need for efficient use of few synaptic vesicles.

Modern consumers realize that recycling is a good practice but that reusing products is even better. Scientists have long debated the relative contributions of vesicle recycling and reuse after neurotransmitter release as well. In the classic mechanism, dubbed 'all-or-none' exocytosis (**Fig. 1a**), a synaptic vesicle fuses with the presynaptic membrane and releases its contents into the synapse; the vesicle membrane is then recycled. Alternately, a synaptic vesicle can form a transient fusion pore in the presynaptic membrane and release only part of its contents; in such 'kiss-and-run' exocytosis, the vesicle is then reused. In this issue, Staal and coworkers[1] clearly establish that small synaptic vesicles in dopaminergic neurons almost exclusively use the kiss-and-run mechanism of exocytosis.

Exocytosis is almost universally accepted as the primary means of chemical communication between neurons. Electron microscopy of freeze-fractured tissue has captured 'omega' structures at the surface of stimulated neurons[2]. Vesicular contents are released in concentration ratios that reflect their stored amounts[3]. Single-cell capacitance changes during exocytosis, indicating an increase in the cellular membrane area[4]. Finally, discrete packets of released chemicals can be detected with amperometry[5], in which the number of easily oxidized molecules released from a vesicle is measured with carbon-fiber microelectrodes.

Much of the current view of exocytosis from small synaptic vesicles has been extrapolated from results obtained with large, dense-core vesicles. Cells with large vesicles primarily use all-or-none exocytosis, and only occasionally kiss-and-run. All-or-none exocytosis of large vesicles is supported by whole-cell capacitance measurements[4] as well as by amperometry, which shows that the amount of transmitter released from a variety of cell types corresponds well with known intravesicular amounts[6]. Video microscopy of chromaffin

cells loaded with a fluorescent dye that accumulates in vesicles also illustrates all-or-none exocytosis: the fluorescent vesicles can be seen to approach the plasma membrane and then completely lose their fluorescence[7] (**Fig. 1a**).

The kiss-and-run mechanism was clearly shown in mast cells by capacitance measurements with a patch-clamp electrode and simultaneous amperometry[8]. The correlated data showed that increases in membrane area are not always accompanied by full extrusion of the vesicle contents. In a study using fast-scan cyclic voltammetry, an intermediate 'kiss-and-hold' state was induced in both mast cells and chromaffin cells by increasing the osmolarity of the extracellular solution[9]. This removed the normal osmotic gradient between the vesicle interior and the extracellular space, preventing efflux of the intravesicular contents.

The applicability of principles derived from large synaptic vesicles to small vesicles in neurons has been unclear. Theoretical considerations predicted that the modest surface tension changes that accompany fusion of a small synaptic vesicle would increase the likelihood of the kiss-and-run mode[10]. However, the restricted population of small vesicles within neurons—along with their extremely small size (diameters of 50 nm or less) and the limited number of molecules they contain—have made it very difficult to experimentally characterize the mode of small-vesicle exocytosis. Traditional capacitance measurements have been unsatisfactory because the fusion of a small surface-area vesicle has been undetectable. Only recently did Klyachko and Jackson manage to drastically reduce the noise level, enabling them to detect the fusion of secretory vesicles similar in size and shape to small synaptic vesicles using capacitance measurements[11]. In this proof-of-concept study, estimates of the fusion pore diameter were smaller than the neurotransmitter molecules that pass through the pore. Nevertheless, this technique now shows promise for the study of small vesicles.

Fluorescence imaging of single neuronal vesicles is also difficult because the size of synaptic vesicles is smaller than the diffrac-

tion limit of standard fluorophore emission wavelengths. Furthermore, fluorescence changes are difficult to interpret because they do not necessarily correlate with the neurotransmitter efflux[12]. However, Gandhi and Stevens[13] used a photobleaching process to show small but discernable differences in fluorescence traces of individual dye-loaded vesicles that suggested kiss-and-run accompanying other forms of exocytosis. Direct efflux experiments are also difficult because neuronal vesicles typically contain only 3,000 to 30,000 neurotransmitter molecules, far fewer than the million or so contained in the large vesicles described above. Amperometry, however, can be used to detect even such a small number of molecules, and it allows real-time measurement of exocytotic events.

Staal and colleagues have now clarified the situation by using amperometry with carbon-fiber microelectrodes to directly monitor exocytosis of endogenous dopamine from cultured ventral midbrain neurons[1]. Measuring K+-stimulated dopamine release from individual neurons, the authors identi-
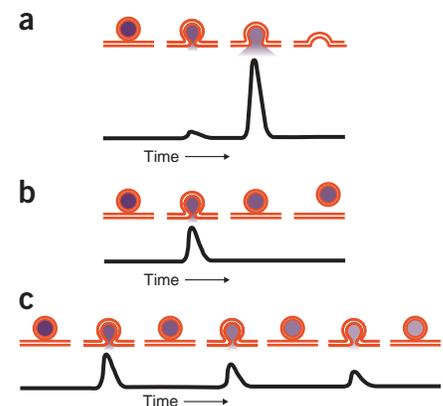
Figure 1 Exocytotic mechanisms for small synaptic vesicles. Schematic drawings of the mechanisms (above) and amperometric current traces (below). (**a**) Full fusion of a small synaptic vesicle after initially forming a small fusion pore. After secretion, the vesicle is temporarily incorporated into the plasma membrane. (**b**) A simple kiss-and-run event. (**c**) A complex kiss-and-run event with three subunits, each decreasing in amplitude.

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fied two classes of release events. In simple events, all the dopamine released from a given synaptic vesicle-membrane fusion site was measured in a single amperometric peak. In complex events, the dopamine released from a given synaptic vesicle-membrane fusion site was measured as a series of discrete peaks. The authors' interpretation is that a simple event consists of a small synaptic vesicle generating a fusion pore in the presynaptic membrane, partially discharging its contents into the synaptic cleft, and then disconnecting from the membrane (**Fig. 1b**). A complex event occurs when the fusion pore flickers rapidly between an open and closed form, allowing repeated partial release of vesicle contents (**Fig. 1c**). Comparison of the amperometric traces from simple and complex events supports this interpretation: the number of dopamine molecules oxidized in a simple event is roughly equivalent to the number of dopamine molecules oxidized in the first sub-unit of a complex event. Thus, both simple and complex events seem to reflect kiss-and-run exocytosis.

The authors found that small synaptic vesicles in midbrain dopaminergic neurons undergo kiss-and-run exocytosis almost exclusively. Kiss-and-run exocytosis is advantageous because it leads to increased longevity of a synaptic vesicle, thereby decreasing the importance of the relatively slow process of vesicle recycling through the endosomal compartment. The authors suggest that such efficient vesicle use is necessary because of the relatively small number of synaptic vesicles present in these midbrain neurons. Kiss-and-run exocytosis also avoids inefficient use of dopamine at synapses that lack well-defined active zones, as is typical of dopaminergic neurons.

The complex form of kiss-and-run may represent a particularly economical form of exocytosis, which may be advantageous if transmitter-loaded vesicles are in short supply. To test this hypothesis, the researchers exposed the cultured neurons to pharmaco-logical agents affecting the secondary messengers that regulate synaptic vesicle cycling. On addition of a phorbol ester, an agent that increases the number of releasable synaptic vesicles, amperometric traces revealed a relative decrease in the number of complex events from 20% to 6%. After inhibition of protein kinase C, reducing the number of releasable vesicles, the total number of exocytotic events per stimulus was decreased by 82%, but amperometric traces showed a relative increase from 20% to 40% in the number of complex events. Thus complex events appear to be favored when fewer releasable vesicles are available.

If the nature of exocytotic mechanisms is determined by the number of vesicles and the nature of the synapse, comparison of the new data[1] with similar data collected from cells with large dense-core vesicles[8] should reveal significant variation. Kiss-and-run occurs in both cases, but there are some notable differences. First, the amperometric trace subunit duration is approximately 200 times shorter in small synaptic vesicles. Second, the fusion pore flickering occurs with a ten-fold increase in frequency in small synaptic vesicles compared to the large dense-core vesicles. Third, the small synaptic vesicles release 25–30% of their dopamine cargo with each flicker of the fusion pore, whereas the large dense-core vesicles release <1% of their dopamine. Clearly, although the same exocytotic mechanism is at work, the fusion pore flickering characteristics are greatly influenced by the size of the vesicle and the function of the cell.

Some questions remain. The new research[1] suggests that kiss-and-run exocytosis is driven by the need for efficient use of a relatively small number of synaptic vesicles. This hypothesis can be further tested by measuring the relative number of full fusion and kiss-and-run events at presynaptic terminals in neurons with a larger number of vesicles, and in neurons that use other neurotransmitters. Amperometry can only detect easily oxidized neurotransmitters such as dopamine, so new strategies will need to be developed for other transmitters such as glutamate. Extracellular calcium is central in regulating exocytosis and release probabilities, and it will be fascinating to explore its influence on the characteristics of kiss-and-run and the flickering pore. Ideally, this would entail amperometric measurements and simultaneous calcium imaging. Because the small synaptic vesicles release such a large percentage of their total neurotransmitter concentration with each flicker of the fusion pore, it would also be interesting to manipulate the intravesicular contents to see whether the kiss-and-hold state can be induced in neurons. Large vesicles forced into a kiss-and-hold state through increased extracellular osmotic pressure undergo massive release when returned to isotonic conditions. Will similar manipulations force the small synaptic vesicles from kiss-and-run to full fusion exocytosis? Future work will tell us whether non-dopaminergic neurons also use a nearly exclusive kiss-and-run mechanism of exocytosis and will explore the implications of kiss-and-run vesicle re-use for the synaptic vesicle recycling mechanism.

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Stiffening the spines

The ability of dendritic spines to change shape in response to synaptic activity is crucial for synaptic plasticity. This motility is regulated by αN-catenin, report Abe *et al.* on page 357. Overexpression of αN-catenin (green; red is PSD95) stabilized spines in cultured neurons, reducing turnover and thereby increasing their number. Lack of αN-catenin increased spine motility, even at established synaptic contacts. Spine αN-catenin was regulated by synaptic activity: blocking activity with tetrodotoxin reduced αN-catenin staining (and increased spine motility), whereas blocking inhibitory neurotransmission increased αN-catenin. The catenins link cadherin cell adhesion molecules to the cytoskeleton, so αN-catenin is well placed to regulate spine dynamics.

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