Synaptic Vesicles Caught Kissing Again

In this issue of *Neuron*, Harata et al. use a novel quenching technique to provide compelling evidence that kiss-and-run is the dominant mode of vesicle fusion at hippocampal synapses and that the prevalence of kiss-and-run can be modulated by stimulus frequency. The increased incidence of kiss-and-run at lower frequencies may ensure that vesicles are available for use during periods of high demand.

The life of a synaptic vesicle is not a simple one. Because only a fraction of the many vesicles that are crowding around small CNS synapses are working on a regular basis, there is a lot of pressure on the 30-or-so active participants to be speedy and efficient (Harata et al., 2001). Recently exocytosed vesicles must quickly return to the interior, refill with neurotransmitter, dock, and prime for re-release in order for the synapse to maintain the ability to signal to its downstream neighbors at even moderate sustained firing rates. While racing back around to the front of the line, these vesicles have the added burden of restoring the full complement of their dizzying array of special proteins and lipids. Does life have to be this hard? A mode of vesicle cycling commonly referred to as "kiss-and-run" just might make it easier by allowing vesicles to open briefly to release their contents but otherwise remain intact at the release site, needing only to be refilled with transmitter prior to reuse. Contention remains in the field, however, as to the significance, and even existence, of this appealing option. In this issue of *Neuron*, a new study from Richard Tsien's lab (Harata et al., 2006) indicates that kiss-and-run is not only taking place, but is much more common than generally appreciated.

Previous support for kiss-and-run at small CNS synapses emerged from imaging studies employing recently developed fluorescent lipophilic FM dyes (Cochilla et al., 1999) and enhanced green fluorescent protein (EGFP) targeted to synaptic vesicles (Miesenbock et al., 1998). One of the first clues came from the Tsien lab's observation that different FM dyes that had been loaded into vesicles were released from active synapses at different rates, indicating that the interior of at least some exocytosing vesicles was exposed to external solution for insufficient time to permit complete release of slower-departitioning dyes (Klingauf et al., 1998). Next came a pair of papers identifying conditions that promoted release of neurotransmitter without concomitant release of dye (Pyle et al., 2000; Stevens and Williams, 2000). More recently, reports have come in that individual vesicles may cycle through multiple rounds of fusion before losing their entire content of FM dye (Aravanis et al., 2003; Richards et al., 2005) and may be exposed to the exterior for as little as 400 ms during fusion (Gandhi and Stevens, 2003). A number of key questions remain, including what fraction of fusion events occur via kiss-and-run, whether this fraction can be modulated, and whether vesicles that fuse via kiss-and-run are more rapidly available for reuse than those that fuse fully. In their current study, Harata et al. address these questions by using novel fluorescence quenching techniques employing bromophenol blue (BPB).

In the presence of extracellular BPB, the rate at which fluorescence decayed from FM1-43-loaded synapses during stimulation was markedly accelerated compared to control experiments performed in the absence of BPB, indicating that BPB was able to rapidly enter vesicles that opened only briefly during kiss-and-run fusion, and to quench the fluorescence of the FM dye that remained trapped within. That is, BPB, by virtue of its small size (comparable to glutamate) and hydrophilic nature, had enough time to get in, even though the FM dye did not have enough time to get out. A critical control experiment showed that there was no change in the fluorescence-decay kinetics of synapses loaded with FM4-64, a red FM variant that is not quenched by BPB, reassuring us that effects of BPB were not due to changes in vesicle releasing or recycling. The difference in the rate of fluorescence decay in the presence versus the absence of BPB was greater when neurons were stimulated at 1 Hz than at 30 Hz, indicating that the kiss-and-run was inhibited at the higher frequency.

A second novel technique was developed to characterize the effects of stimulation frequency on kiss-and-run in more detail: BPB quenching of EGFP targeted to the intraluminal domain of synaptic vesicles (Miesenbock et al., 1998). For these EGFP experiments, Harata et al. treated their neurons with the proton-pump inhibitor, bafilomycin, to abolish fluorescence-quenching acidification of vesicles and ensure that only EGFP exposed to the BPB-containing extracellular solution would be quenched. Under these conditions, the rate at which EGFP fluorescence decayed reported the rate at which vesicles first fused with the membrane during a bout of stimulation. By comparing the kinetics of FM fluorescence decay and EGFP/BPB-monitored first fusion during long trains of stimulation, Harata et al. were able to estimate that over 80% of all fusion events occurred via kiss-and-run at 0.3 Hz, dropping to about 50% at 30 Hz. Further analysis showed that both kiss-and-run and full-fusion could occur at the same synapse and that all cycling vesicles were capable of kiss-and-run, not just those that made up the readily releasable pool.

What role might kiss-and-run play in determining the rate at which vesicles become available for reuse? Harata et al. found that the rate of vesicle reuse increased ~10-fold when stimulation frequency increased 100-fold (0.3 Hz to 30 Hz), but much of the increase in the rate of reuse can almost certainly be attributed to the increased rate of release as stimulation frequency rose. The estimated half-time for reuse was ~10 s at 30 Hz. Although Harata et al. interpret this as a significant advantage over the several-tens-of-seconds previously estimated to be required for complete recovery from

full fusion, Fernandez-Alfonso and Ryan (2004) recently reconsidered the FM studies that gave rise to the previous estimate and suggested an alternative interpretation of the data that would significantly reduce estimates of the time required for endocytosis of fully fused vesicles. In addition, a recent EGFP study (Li et al., 2005) found no evidence for preferential reuse of recently exocytosed vesicles, consistent with morphological studies showing that recently exocytosed vesicles were only slightly more likely than other vesicles to be found close to the active zone (Schikorski and Stevens, 2001). Although a kiss-and-run vesicle that remains on the surface for ~1 s would seem to have a clear temporal advantage over a full-fusing one that lingers for ~10 s (Gandhi and Stevens, 2003), the current results do not provide strong direct support for the hypothesis that kiss-and-run serves as an effective mechanism to speed up the rate at which vesicles become available for reuse, especially at high frequencies, when it might be expected to do the most good, and further work will be required to resolve this issue.

Another big question that still awaits an answer is whether kiss-and-run fusion affects neurotransmitter release from individual vesicles. It is an intriguing notion (and common perception) that kiss-and-run could alter the amount or time course of neurotransmitter released by a fusing vesicle, but there is little reason to think that the mode of fusion has any effect on quantal size. Direct electrophysiological measurements of miniature excitatory postsynaptic potentials under conditions that increased the likelihood of kiss-and-run fusion showed no reduction in amplitude or slowing of kinetics of unitary responses (Stevens and Williams, 2000). Although much depends on the exact characteristics of the still-undefined fusion pore, modeling estimates based on reasonable values indicate that the 400 ms time course of the briefest kiss-and-run events (Gandhi and Stevens, 2003) is well over the upper limit of ~1 ms thought to be required for complete emptying of a glutamate-filled synaptic vesicle (see Richards et al., 2005). The possibility remains that transmitter diffuses so slowly from a kiss-and-run fusion pore that it serves only to desensitize postsynaptic receptors without ever activating them, but there is no direct evidence to date that supports this hypothesis. Further testing under conditions that promote or suppress kiss-and-run, perhaps employing cyclothiazide, a drug that blocks glutamate receptor desensitization, or γ-DGG, a glutamate receptor antagonist that can be used to assay transmitter concentration in the synaptic cleft, should resolve this issue in the future.

Harata et al. propose that kiss-and-run serves to conserve resources during periods of low-frequency firing and ensures that hippocampal synapses are ready to take on the demands of high-frequency activity with a full quota of fusion-competent vesicles. Whether or not this turns out to be its functional role, the current study significantly extends our appreciation for kiss-and-run as the dominant mode of fusion at these synapses and identifies frequency of stimulation as a factor that can modulate the prevalence of kiss-and-run. In addition, Harata et al. have provided researchers in the field with powerful new tools to address those issues that remain unresolved.

Jane M. Sullivan[1]
[1] Department of Physiology and Biophysics
University of Washington School of Medicine
Box 357290
Seattle, Washington 98195

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Flick-Induced Flips in Perception

Microsaccades are miniature eye movements produced involuntarily during visual fixation of stationary objects. Since their first description more than 40 years ago, the role of microsaccades in vision has been controversial. In this issue, Martinez-Conde and colleagues present a solution to the long-standing research problem connecting this basic oculomotor function to visual perception, by showing that microsaccades may control peripheral vision during visual fixation by inducing flips in bistable peripheral percepts in head-unrestrained viewing. Their study provides new insight into the functional connectivity between oculomotor function and visual perception.

Information processing of stationary visual targets is restricted to periods during which the eyes appear to be at rest, a state called *visual fixation*. However, fixation is in reality a highly dynamic oculomotor process featuring a surprising panoply of involuntary and unconscious miniature eye movements. In the 1950s, experimental suppression of these movements in a paradigm called *retinal stabilization* was reported to induce *perceptual fading* (Ditchburn and Ginsborg, 1952; Riggs et al., 1953), leading to the hypothesis that the three major "fixational" eye movements—microsaccades, drifts, and tremors—are generated actively by the oculomotor