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Slow or fast? A tale of synaptic vesicle recycling

A new model accounts for synaptic transmission speed

By Shigeki Watanabe*

Whether resting on a couch, walking in a park, or running in a race, our nervous system must sustain an activity commensurate with physical and mental demands. Neuronal communication is carried out by chemical signals that are released from one nerve terminal and received by another. These chemical signals, or neurotransmitters, are packaged in vesicles. When neurons are activated, some of these vesicles fuse with the cell membrane—releasing the neurotransmitters to be detected by interconnected neurons.

During sustained neuronal activity, this process is repeated over and over, which imposes three major challenges on neurons. First, vesicle membrane is continuously added to the cell surface. Second, vesicle proteins become lodged at the site of fusion. Third, the supply of synaptic vesicles in the terminal becomes scarce. To counteract these changes,

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synaptic vesicle membrane and proteins are rapidly removed from the plasma membrane by local recycling. This local recycling generates the new synaptic vesicles that are then loaded with neurotransmitter for further rounds of fusion and release.

TWO MODELS FOR SYNAPTIC VESICLE RECYCLING. In the early 1970s, two models were put forward for synaptic vesicle recycling that were based on the analysis

of frog neuromuscular junctions by electron microscopy: “kiss and run” (1, 2) and clathrin-mediated endocytosis (3, 4). These two models resemble two methods used in the recycling of glass bottles. The kiss-and-run mechanism is like refilling the same bottle: The same vesicle that has just undergone fusion is retrieved and refilled (see the figure, panel A). The clathrin-mediated mechanism is like molding a new bottle from a used one: the old vesicle is resorbed into the plasma membrane and must be subsequently remolded (figure, panel B).

Speed is a factor that distinguishes these two models. The kiss-and-run pathway is fast, requiring only 1 to 2 s (5). By contrast, in the clathrin model, the remodeling of a

new vesicle requires ~10 to 20 s (3, 4, 6–8). Given the importance of speed to neuronal communication, the kiss-and-run model may seem more plausible, yet many studies have pointed to the essential function of clathrin and clathrin-associated proteins in synaptic vesicle recycling (9, 10). Does clathrin function suffice under all conditions? Or does kiss-and-run recycling predominate in some conditions—perhaps during periods of prolonged stimulation? Despite extensive research in the last 40 years, there are no definitive answers to these questions.

A TECHNIQUE THAT CAPTURES MEMBRANE DYNAMICS. One problem with previous studies is that they used intense stimulation to guarantee the observation of endocytic structures. To observe synaptic vesicle recycling after a single stimulus or a short burst of stimuli, we used lentivirus to express the light-sensitive channelrhodopsin ChetaTC in mouse hippocampal neurons. These channels act like a switch for neuronal activity: A short flash of light activates the channelrhodopsin, which switches the neurons “on” and causes fusion of synaptic vesicles at the terminals. The neurons were then frozen at defined time points after stimulation. By controlling the interval between the stimulation and freezing, we were able to capture snapshots of the events at different time points—in effect, generating a “flip-book” of membrane movement during synaptic transmission with millisecond temporal resolution (11).

RAPID REMOVAL OF VESICLE MEMBRANE. According to both the clathrin and the kiss-and-run models, membrane invaginations equal to the size of a synaptic vesicle

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GRAND PRIZE WINNER: Shigeki Watanabe

Shigeki Watanabe received his undergraduate and Ph.D. degrees from University of Utah. For his Ph.D. and postdoctoral work, he has been studying

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FINALIST: Julija Krupic

Julija Krupic received her undergraduate degree from Vilnius University and a Ph.D. from University College London (UCL). She is currently a Sir Henry Wellcome Fel-

low at UCL, where she conducts research on how place cell activity guides animal behavior. In 2016, Julija will move to the Salk Institute where she will study how connectivity affects the functional properties of place cells.

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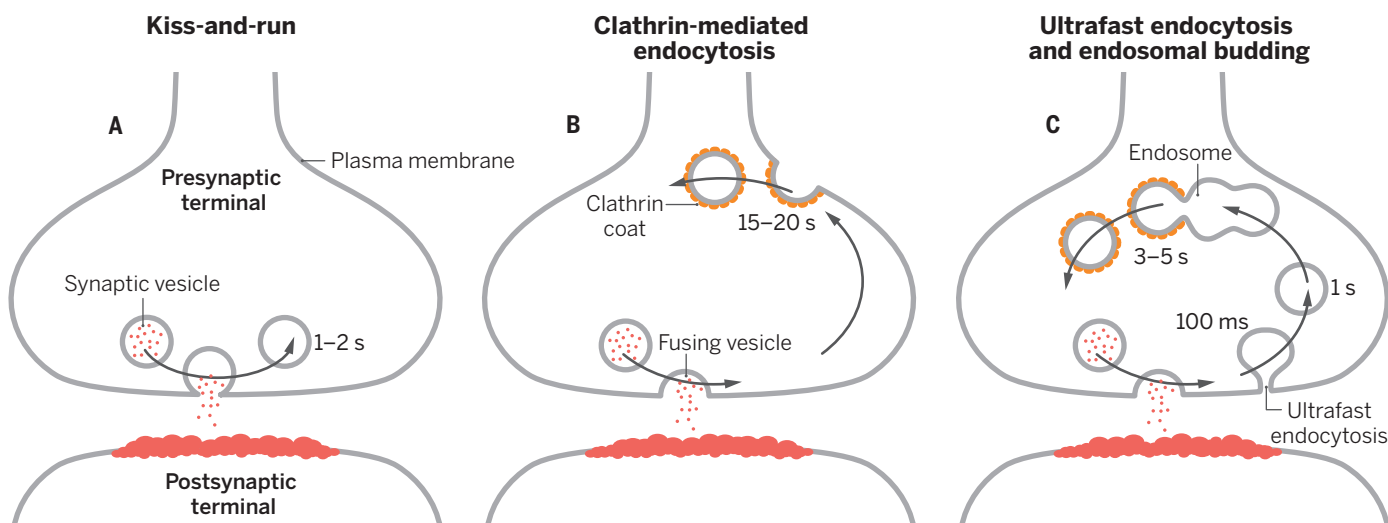


FINALIST: Jeremiah Cohen

Jeremiah Cohen received his undergraduate degree from Brandeis University, a Ph.D. in neuroscience from Vanderbilt University, and completed his

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Models of synaptic recycling. (A) The kiss-and-run model. Vesicles transiently fuse with the plasma membrane. After the neurotransmitter release, the fusion pore is closed and the vesicles are recovered. (B) The clathrin-mediated endocytosis model. A synaptic vesicle fuses and collapses into the membrane. A new vesicle is formed in a region distant from the fusion site. (C) A new model of synaptic vesicle recycling. After a rapid internalization of the membrane via ultrafast endocytosis, the vesicle membrane is delivered to an endosome. Clathrin-mediated regeneration of synaptic vesicles occurs at the endosome.

would be expected to form on the plasma membrane after fusion of synaptic vesicles. However, we observed invaginations roughly twice as large as a synaptic vesicle in the region immediately lateral to the site of the fusion (12, 13). This process was initiated 50 ms after stimulation and was completed within 100 ms, far faster than either the kiss-and-run or clathrin-mediated mechanisms. Unlike what would have been expected on the basis of the clathrin or kiss-and-run models, however, we observed that a large portion of membrane is taken into the terminal, and synaptic vesicles are not recovered directly from the plasma membrane (figure, panel C).

REMOLDING VESICLES FROM ENDOSOMES. To determine how vesicles are regenerated, we followed the fate of the internalized membrane using ferritin as a tracer (14). About 1 s after stimulation, the endocytosed membrane was delivered to another organelle, an endosome. Clathrin-coated buds about the size of a synaptic vesicle formed on these endosomes 1 to 3 s after stimulation. Synaptic vesicles containing ferritin molecules accumulated in the terminal after 5 to 6 s. When clathrin function was genetically or pharmacologically blocked, ferritin molecules were retained in endosomes. These results suggested that synaptic vesicles are reconstituted at endosomes and that this process is dependent on the clathrin function (14). Thus, the vesicles are molded anew; however, the molding takes place within the cell rather than at the cell surface (figure, panel C).

TOWARD A UNIFIED MODEL? The recycling mechanism seems to have an ultrafast component followed by a slower component (figure, panel C). The ultrafast mechanism rapidly clears the fusion sites of excess membrane (and presumably of vesicle proteins). The slower mechanism, a clathrin-dependent process, reconstitutes vesicles at a more leisurely pace from endosomes. Our research suggests that the ultrafast component of vesicle recycling is not a kiss-and-run method and that the

“These results suggested that...the vesicles are molded anew; however, the molding takes place within the cell rather than at the cell surface.”

slow mechanism does not operate at the plasma membrane. What could account for the disagreement between our data and data reported in previous publications?

The answer to this question may lie in the experimental conditions. Mice are endotherms, yet many of the previous experiments were conducted with tissue kept at room temperature. When we repeated our experiments at room temperature, synaptic vesicles were regenerated directly from the plasma membrane in a clathrin-dependent fashion (14).

What about kiss-and-run? Although we

did not observe kiss-and-run structures, these results do not rule out that this process may be occurring at the nerve terminals, because our technique is not sensitive enough to determine whether the fusion pore of a particular vesicle is stable over time. It is also possible that synapses have adapted different mechanisms to meet specific demands for recycling.

Ultrafast endocytosis comes as a bit of a surprise. Why do synapses need to remove membrane from the surface so rapidly? One possibility is that the immediate role for endocytosis is not to restore vesicle numbers but rather to clear the fusion site so that another vesicle can fuse in rapid succession.

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