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"Kiss-and-Run" Rules the Inner Lives of Neurons

Neurons transmit chemical signals in a fleeting “kiss-and-run” process, which in large part determines how quickly neurons can fire, according to new studies by Howard Hughes Medical Institute researchers.

The transfer of information between nerve cells occurs when chemicals called neurotransmitters are released into the synapse, the junction between neurons. Electrical impulses in the neuron cause tiny vesicles loaded with neurotransmitters to move to the tip of the nerve terminal where they are released.

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In an article published in the June 5, 2003, issue of the journal *Nature*, HHMI investigator Charles F. Stevens and Sunil Gandhi, both at The Salk Institute, reported that they have devised a technique that permits them to visualize individual vesicles after they have released their cargo. The new findings are significant, said the researchers, because they answer questions about the rate at which synaptic vesicles can be recycled. This rate determines how much information nerve cells can transmit.

Stevens and Gandhi have identified three distinct ways in which a used vesicle can be retrieved from the surface of the nerve cell once it has released its cargo. The fastest of these, called the “kiss-and-run” mode, takes less than a second; the slower “compensatory” mode takes up to 21 seconds; and the “stranded” mode leaves the vesicle stuck at the surface until the next nerve impulse triggers its retrieval.

According to Stevens, the latest findings settle lingering questions about how vesicle retrieval occurs. Early electron microscopy images of vesicles in synapses were interpreted as either a kiss-and-run model or one in which the vesicle is completely incorporated into the cell membrane, to be drawn back

into the cell.

“The advance that we have made is to figure out a way of imaging individual vesicles so that we can measure the time course of single-vesicle events and immediately answer these questions,” said Stevens.

The optical recording technique devised by Stevens and Gandhi involves genetically modifying a gene for one type of vesicle protein to incorporate a special form of green fluorescent protein. This modified fluorescent protein, developed by other researchers, does not fluoresce under acidic conditions normally present in vesicles fully loaded with neurotransmitter. However, when the vesicle releases its payload, the interior becomes less acidic and the vesicle glows a bright green.

Thus, said Stevens, by imaging individual vesicles in cell cultures of neurons, it is now possible to detect how and when vesicles release their cargo at the synaptic membrane.

“Among the minor observations we made was that vesicles can re-acidify themselves in less than half a second,” said Stevens. “We also observed that the proteins in the vesicle are maintained together, so that when a vesicle is taken back in from the membrane, the same proteins are still there, even if the vesicle had been fused with the membrane for quite a while.

“And the third thing that was surprising is that all vesicles across different preparations have basically the same number of these tagged protein molecules,” said Stevens. “This means that they are either saturated or there is some mechanism for counting the proteins.”

The major observations from their studies, said Stevens, are that there are three modes of vesicle release and retrieval from the membrane. “One is what you could call classical, when the vesicle opens to the outside world, stays open for about eight seconds, and then is taken back in at random times extending out to twelve or fourteen seconds,” he said. This finding confirms previous theories about modes of vesicle recycling, he said.

“However, sometimes if the vesicle failed to be re-internalized to be reused again by about fourteen or fifteen seconds, sometimes it got stuck there,” said Stevens. In this “stranded” mode, the vesicle remained stuck until another nerve impulse caused it to be zipped into the interior of the neuron to be recycled. Presumably, stranding occurs because vesicle recycling depends somehow on the level of calcium in the nerve cell, which rises precipitously during a nerve impulse, and drops afterward, said Stevens.

“The third recycling mode we observed was a kiss-and-run-mode that happened very rapidly, in less than half a second,” said Stevens. “Also, we showed experimentally that in this mode there was a ‘fusion pore’ formed where the vesicle contacted the membrane,” he said.

Stevens and Gandhi also found that vesicles appear to adjust their mode of recycling based on the probability that a given synapse will trigger the release

of a vesicle's cargo. Vesicles in synapses with a low-release probability are more likely to use the rapid kiss-and-run mode, he said, while those vesicles in a higher-probability synapse use the slower compensatory mode.

Future studies will seek to determine the molecules responsible for recycling and how structures such as the fusion pore form. The researchers will also explore the role of calcium in recycling, as well as the advantages to the nerve cell of using the kiss-and-run recycling mode.