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Reconsidering Dynamin's Role in Nerve Cell Function

In just one-thousandth of a second, a neuron can dump up to 5,000 molecules of its chemical messenger, a neurotransmitter, into the synapse, where it will trigger an impulse in its neighboring nerve cell. Neurons are constantly engaged in chemical conversations with their neighbors, but the volume of this chatter can put a real strain on the production lines that package neurotransmitters.

Yet knowing what the components of those production lines are and what each does is vital to building a better understanding of how the brain works. As a case in point, the enzyme dynamin 1 has long fascinated scientists because it was thought to play an essential role in helping to package neurotransmitters for release. The secretion of neurotransmitters is mediated by the fusion of small neurotransmitter-filled vesicles (synaptic vesicles) with the cell outer membrane (plasma membrane). New studies by Howard Hughes Medical Institute researchers at Yale University have shown that under certain conditions, however, dynamin 1 may not be needed for basic nerve cell function.

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— **Pietro De Camilli**

Their study has found that dynamin 1, which is a major component of the machinery that severs the necks of hollow bud-like invaginations of the cell membrane as new vesicles form, is only important when neurons are stimulated to high levels of activity. This new information offers intriguing insight into a fundamental cellular process called endocytosis, which is

responsible for creating new synaptic vesicles and thus for neurotransmission. Endocytosis is also critical for internalization of extracellular material into all cells.

Howard Hughes Medical Institute investigator Pietro De Camilli and his Yale colleagues reported their discovery in the April 27, 2007, issue of the journal *Science*. Co-authors of the study were from Weill Medical College of Cornell University; The Rockefeller University; the FIRC Institute for Molecular Oncology Foundation and Università Vita—Salute San Raffaele in Italy; and the University of Colorado.

There has been a tremendous amount of interest in how this protein works—whether it acts directly on the membranous neck of budding vesicles, or perhaps regulates the recruitment of other proteins to this site, said De Camilli. Dynamin 1's confinement to and abundance in the brain—as opposed to the two other forms of dynamin found in mammals—had led scientists to suspect that it might play an essential role in forming synaptic vesicles. Since it was assumed that dynamin 1 was so critical, nobody bothered to test the effects of totally knocking it out, said De Camilli. It was believed that such animals would not even begin to develop a functional nervous system. But we figured that nature never stops surprising us, so we decided such an experiment was worth the investment.

So the researchers tested the effects of totally knocking out the dynamin 1 gene in mice. Much to our surprise, the animals developed normally, and their nervous system could support neurotransmission in the absence of dynamin 1, first author Shawn Ferguson and a member of the De Camilli laboratory said. Immediately after birth, the animals without dynamin 1 behaved just like normal mice. In later days, however, the mice did begin to lose motor coordination and eventually died.

In their experiments, the researchers were seeking to understand the precise conditions under which dynamin 1 was necessary for the reformation of synaptic vesicles. They found that the baseline level of neurotransmission was normal in the knockout mice. However, the animals' neurons could not operate under conditions of prolonged strong excitation. Under those conditions, the neurons completely stopped transmitting impulses, recovering only slowly after excitation ceased.

The researchers also took a close look at the synapses in the knockout mice using electron microscopy. The big surprise was that there were a lot of synaptic vesicles that formed without dynamin 1, said De Camilli. But we also saw that a lot of the buds were not able to separate from the plasma membrane. They were interconnected and formed a branched tubular network that nobody had seen before.

The microscopy studies also showed a considerable variation in the size of synaptic vesicles in neurons from the knockout mice which was matched by a corresponding variability in their neurotransmitter content as detected by electrophysiological studies, noted De Camilli. Further studies revealed that synaptic vesicle recycling could not keep up with the demands of high

frequency nerve stimulation while efficient endocytosis occurred under resting or low frequency stimulation conditions, he said. Significantly, the researchers could restore endocytosis by reintroducing dynamin 1 or by overexpressing the normally much less abundant dynamin 2 or 3 into the knockout animals' nerve cells, said De Camilli.

A main message of this paper is that dynamin 1 is not needed for basic nerve cell function, and that the tremendous amount of dynamin 1 that exists in the brain appears necessary to increase the adaptability of synaptic function to stimulation, concluded De Camilli. Since the fruitfly *Drosophila* has only one dynamin gene, while mammals have three, the existence of dynamin 1 might enhance the plasticity of the synapse.

This finding also raises the question of whether dynamin is truly essential for endocytosis, he said. He and his colleagues are now exploring the function of the other forms of dynamin in neurotransmission in more detail.