Breakthrough technology observes synapse in real time, supporting theory of vesicular recycling

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For the first time, scientists at Weill Cornell Medical College in New York City have observed in real time a cellular mechanism that's crucial to how brain cells communicate.

In doing so, they've also laid to rest a competing theory as to how key cellular processes -- called endocytosis and exocytosis -- work.

The scientists published their findings in this week's online edition of *Proceedings of the National Academy of Sciences* (Dec. 18 print edition).

Healthy neurological function hinges on the efficient passage of information between brain cells via the synapse, and exocytosis/endocytosis is the complex trafficking mechanism that allows this to happen.

At its simplest level, exocytosis involves the packaging, transport and delivery of neurotransmitter chemicals in sac-like structures called vesicles. These vesicles move from the interior of the cell to the cell membrane, where they deliver their information-rich cargo to the synapse. Endocytosis involves a similar function in the reverse direction, with incoming vesicles being transported into the cell's interior.

The vesicles aren't discarded, however. Instead, once they release their cargo they are recycled for use in another go-round. There have been two competing theories about how that recycling occurs -- either the vesicle fragments upon delivering its cargo and must be rebuilt, or it simply empties itself like milk from a bottle which is then resealed.

"The vast bulk of the evidence suggests the former theory is actually the correct one, but it's been tempting to think of the 'resealable spout' theory, because it seems so logical and because there's been some ambiguous evidence that it might be true," says the study's co-author Dr. Timothy Ryan, professor of biochemistry at Weill Cornell Medical College.

The trouble is, no one had ever found a way to observe -- accurately and in real time -- synaptic vesicle recycling as it occurs.

That has changed with this new paper. "We have taken advantage of recent advances in fluorescent 'tagging' of molecules involved in these cellular processes, as well as new microscopy technologies that give us an incredible new ability to watch all of this, up close and in real-time," says Dr. Ryan.

Specifically, Dr. Ryan used a fluorescent chemical stain called pHluorin and genetically fused it to a vesicular protein called vGlut1. "We've used this fluorescent tagging approach before, but with molecules that can exist on either the outside or the inside of the vesicle," Dr. Ryan notes.

"VGlut1 gives us a much more precise view, since it only inhabits the inside of the vesicle," he adds. "That means that when we see the green fluorescent tag move outside of the vesicle, then the vesicle itself must have ruptured in some way. This gives us a much more accurate picture of the recycling process."

At the same time, the team took advantage of new breakthroughs in optical microscopy that maximize how much of the tag's fluorescent light can be "grabbed" by the microscope. This approach allowed them, for the first time, to follow how individual synaptic molecules are delivered and retrieved from the synaptic surface.

"The result is an accurate view into this hitherto mysterious synaptic phenomenon," Dr. Ryan says.

The "resealable spout" hypothesis of vesicular recycling (also known as the "kiss-and-run" theory) may be the first casualty of this new insight.

"We observed that, although recycling appears to occur within a set but somewhat variable time-frame, it's still using the same mechanism -- the vesicle falls apart upon delivering its cargo to the cell membrane, and then enzymes go to work re-building it for the next cycle," Dr. Ryan adds. "I think this real-time observation really closes the door on the 'kiss-and-run' theory of vesicular recycling."

The new technology used in these experiments should bring scientists much more insight into how the synapse works generally, and that could have real implications for our understanding of neurological health and illness, Dr. Ryan says.

"This is all part of the 'shop manual' of neurological function that we are currently putting together, piece by piece," he says. "Discoveries like these are adding new pages to the manual every day, and it's that kind of knowledge that will someday save, extend and improve lives."

Source: New York- Presbyterian Hospital