NEUROTECHNIQUES

Keep the camera STEDy

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Using a new microscopy technique, researchers observe synaptic vesicle movements in live neurons.

Where do synaptic vesicles go when no one is looking? Researchers typically use fluorescent microscopy and time-lapse imaging to study cellular motion. However, neighboring synaptic vesicles often blur together in standard fluorescent microscopy, making their behaviors difficult to study. Now Westphal *et al.* report a light microscopy technique that distinguishes synaptic vesicles and their movements in live cells in a recent article in *Science*.

The diffraction limit of light microscopy is half the wavelength of visible light (approximately 200 nm), so objects located within 200 nm of each other blur together. Synaptic vesicles are approximately 40 nm in diameter, so several neighboring synaptic vesicles can together resemble one big, bright blob. In contrast, electron microscopy distinguishes small structures, like synaptic vesicles, but requires tissues to be fixed.

In standard fluorescent microscopy, a beam of light excites specific fluorophores used to label proteins or structures of interest. As the fluorophores relax back to their ground state, they emit light, which is filtered and recorded in an image. Stimulated emission depletion (STED) microscopy combines a traditional excitation light beam with a donut-shaped light beam of lower energy. This second light beam actively reduces the energy level of imaged fluorophores, preventing photon emission. In STED, researchers superimpose the excitation beam on the empty center of the depletion donut, so only molecules at the center of the imaged region fluoresce. The authors previously showed that by limiting the diameter of the center of the depletion donut (focal spot), they could break the 200 nm diffraction limit in fixed or stationary samples.

Can STED image physiological events, like vesicle movements, in real time? The authors fluorescently labeled synaptic vesicles in cultured hippocampal neurons. Unlike confocal microscopy, STED microscopy with a focal spot limited to 62 nm distinguished individual vesicles. The authors captured vesicle motion in time-lapse images taken 28 times per second.

Although synaptic vesicles remained stationary most of the time, the authors recorded rapid, non-directional vesicle movements in most traces. Synaptic activity induced by KCl did not affect vesicle mobility. Latrunculin A and nocodazole, which inhibit actin and microtubule polymerization, respectively, reduced but did not prevent vesicle mobility. These data suggest that both active transport and passive diffusion are involved in synaptic vesicle motion.

Where do synaptic vesicles go? The authors summed all of the frames in their traces. Synaptic vesicles remained stationary in 'hot spots' scattered along the axon and occasionally moved in linear 'tracks'. Because cultured hippocampal neurons contain few vesicle docking sites, the authors believe the hot spots are pockets that physically restrain the vesicles.

After the excitation beam bleached vesicles present at the start of a trace, new vesicles entered imaged areas at a rate of 0.5-3 vesicles per second. At this rate, many vesicles continuously pass through synaptic boutons. According to the authors, these data suggest that synaptic boutons within the same neuron may share common pools of vesicles.

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1. Westphal, V. *et al.* Video-rate far-field optical nanoscopy dissects synaptic vesicle movement. *Science* **320**, 246–249(2008). | [Article](#) | [PubMed](#) | [ChemPort](#) |