The Hong Kong University of Science & Technology  
Division of Biomedical Engineering

GUEST SEMINAR

Real-time Three-dimensional Nanometer-Accuracy Tracking of Single Synaptic Vesicles in Live Hippocampal Neurons

by

Prof. Hyokeun PARK  
Division of Life Science and Department of Physics  
The Hong Kong University of Science & Technology

Date  : 15 Oct 2014 (Wed)  
Time  : 2:00 pm  
Venue: Room 5583 (lift 27-28)

Abstract

The position and movement of synaptic vesicles in presynaptic terminals are important for synaptic transmission. However, accurate three-dimensional tracking of single synaptic vesicles in presynaptic terminals has remained a challenge. Using dual focus imaging optics, we have been able to track quantum dot-labeled single vesicles in three-dimensions, with an accuracy of 20 nm in x-y and 30 nm in z in 10 Hz imaging. We observed three typical patterns of movement of single vesicles up to the moment of exocytosis - minimal (almost stationary), intraboutonic, and intersynaptic movement. Intersynaptic movement sometimes appeared as largely unidirectional motion. Using different loading protocols, we tracked the dynamics of synaptic vesicles derived from either readily releasable pool (RRP) or total recycling pool (TRP). The vesicles from RRP were located much closer to fusion sites than those from TRP. This spatial disparity determines the identity of the synaptic vesicles pool. Also two different modes of exocytosis - full-collapse-fusion and kiss-and-run - were distinguished using the degree of quenching of photoluminescence by trypan blue. Vesicles that underwent kiss-and-run traveled shorter distances before fusion than those which underwent full-collapse fusion, which support the idea that the choice between fusion modes is not solely determined at the last moment but depends on prior vesicle state, including starting position before stimulation and pool of origin. In order to determine the location of releasing position relative to the center of the active zone, we localized the centroids of spectrally separable markers, FM 4-64 (presynaptic sites) and PSD 95-GFP (postsynaptic sites) in three dimensions using the same methods as Qdot loaded vesicles. We found that synaptic vesicles undergoing kiss-and-run tend to fuse close to the center of the active zone whereas vesicles undergoing full-collapse fusion tend to fuse all around the synapse. This difference of fusion mode may be related to the difference in the spatial distribution of AMPA and NMDA receptors, implying the relation between fusion mode and synaptic transmission.

ALL ARE WELCOME