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Vesicle Structural Changes Control Content Release of Transmitters and Hormones

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Vesicle fusion releases transmitters, hormones, and peptides to mediate many biological processes crucial to an animal's life, such as immune responses, mood changes, stress responses and synaptic transmission [1-4]. Vesicle fusion is executed via formation of an Ω-shape intermediate structure at the plasma membrane to release its content. Previous studies suggest that the vesicular content release is controlled by the fusion pore through two fusion modes: 1) full-collapse in which the fusion pore dilates till vesicle flattening to facilitate release, and 2) kiss-and-run in which the pore open and close to limit content release. Recent studies challenge this general view. It has been proposed that the vesicle shrink instead of full-collapse into the plasma membrane [5] and that the kiss-and-run may not limit release [6]. However, direct evidence of shrinking is missing; why cells prefer shrinking or full-collapse is unclear; whether and how shrinking facilitates content release are unknown. We are going to answer these questions, visualizing the dynamics of membrane profiles and fusion pores, using advanced techniques, including super-resolution STED microscopy, transmission electron microscopy (TEM) and focused ion beam-scanning electron microscopy (FIB-SEM). Live evidence on primary bovine chromaffin cells showed a direct observation of the Ω-shape membrane profile shrinking. Bovine adrenal chromaffin cells were transfected with EGFP linked to phospholipase C delta PH domain (PH_G). PH_G binds to PtdIns (4,5) P2 (PIP_2) at the plasma membrane (PM) cytosolic-facing leaflet and thus labels the plasma membrane. The cells were bathed with the impermeable dye Atto532 which may enter the fusing Ω-profile to confirm the structural changes revealed by PH_G imaging [6]. We induced vesicle fusion by applying a 1-s depolarization from -80 to +10 mV through a pipette at the whole-cell configuration, which induced calcium currents and capacitance changes reflecting robust exo- and endocytosis. Shrinking was reflected as decreases of both Ω-profile's width and height, resulting in the proportionally reduced Ω-profile in most cases (figure 1). Additional evidence was given using conventional transmission electron microscopy (TEM). Primary bovine chromaffin cells were stimulated with a 90 mM KCl solution, chemically fixed and embedded for electron microscopy ultrathin sections. The screening revealed various sizes of Ω-profiles (figure 2). The width ranged from 56-784nm with larger width correlated with larger height, consistent with various W-profile sizes we observed in live cells. These results together provided direct visualization evidence for shrink-fusion. Establish shrink fusion model and elucidate its underlie mechanism. Primary bovine chromaffin cell culture. Among the cells models that have provided insight in the exocytosis, the adrenal chromaffin cells have taken a prominent place. Chromaffin cells contain large dense-core vesicles (~300nm) and allows the use of recent super-resolution microscopy. The strategy will be the same used to visualize the Ω-profile by super-resolution STED microscopy. Chromaffin cells will be transfected with PH-EGFP, bathed with cell impermeable Atto532, stimulated and imaged by STED. We will be focus on the fusion pore dynamics as PH- Ω changes in size. The goal is to elucidate the pathway, visualizing the dynamic of the profile, from the initial contact with the plasma membrane until its disappearance. The strategy will be to employ the FIB-SEM. The technique is a powerful approach for three-dimensional imaging, that allows the visualization of a whole chromaffin cell with a resolution of 4nm. This resolution is lower than the one provided by the conventional EM (0.2 nm), but it will be sufficient to visualize the Ω-profiles.

The cells will be stimulated with 90 mM KCl solution, fixed and processed for the FIB-SEM. Once the data are collected, we will proceed with analysis and quantification using volume annotation and segmentation software VAST [7]. The goal is to visualize all fusion events in the whole cell and see whether the 3D volumes of the Ω-profiles, are consistent with the ones observed in the live preparation. Vesicle fusion mediates fundamental processes like synaptic transmission and immune responses. Regulation of synaptic transmission plays a pivotal role in many functions of our nervous system, such as neuronal development and learning and memory. Furthermore, in many neurological disorders synaptic transmission is impaired. The proposed work might be the piece of puzzle that thus ultimately improve our understanding on how synaptic transmission is regulated and how these important brain functions and disorders are mediated [8].

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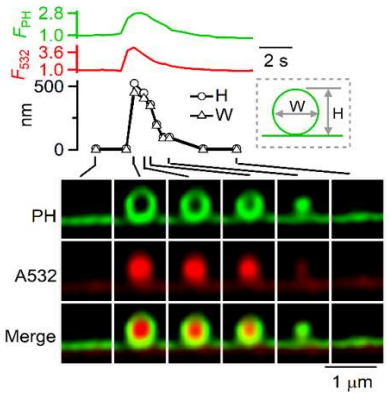

Figure 1. Direct visualization of Ω-shape membrane profile shrinking. PH-Ω fluorescence, A532 spot fluorescence, PH- Ω width (W_{Ω}), PH- Ω height (H_{Ω}) and sampled images at times indicated with lines showing fusion with various patterns of Ω – profile structural changes. The images show the Ω-shape membrane profile shrinking. Images were acquired at the STED XZ/Y_{fix} configuration.

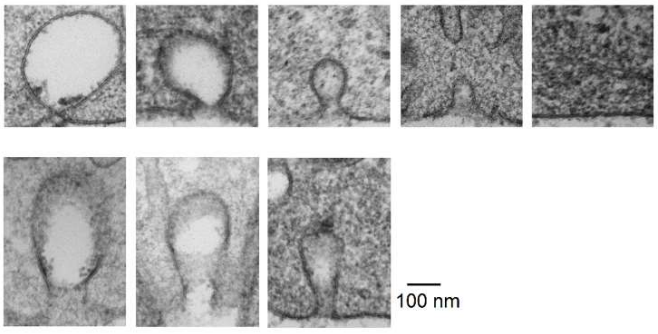

Figure 2. Electron microscopic images of Ω-profiles with different sizes. Cells were stimulated with 70 mM KCl for 90 s.

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