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Investigation of Exocytosis for a New Paradigm of Plasticity in Biological Systems Using Electrochemistry and Mass Spectrometry Imaging

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Abstract

Cellular communication is a vital process and serves as the basis for complex brain functions in multicellular organisms. The majority of cellular communication is achieved via the release of specific signaling molecules, transmitters and modulators, via a process termed exocytosis. Since dysfunction of cellular communication can result in severe outcomes, exocytosis is tightly controlled and regulated. Secretory vesicles are the cellular organelles that serve as functional units to carry out exocytosis. To initiate exocytosis, secretory vesicles need to be docked and primed to the cell membrane. The subsequent fusion between vesicles and cell membrane allows the release of signaling molecules to the extracellular space where they can travel to other cells and transfer a message.

Exocytosis is a highly complicated process and its regulation involves a large number of proteins as well as membrane lipids. To study specific aspects of exocytosis, simplified biological model systems have been developed and widely used, including mammalian cell lines and the invertebrate *Drosophila melanogaster*. A couple of methodologies in the areas of electrochemistry and imaging are available for quantification or visualization of exocytosis. Single cell amperometry (SCA) and intracellular vesicle impact electrochemical cytometry (IVIEC) are two electrochemical techniques that can be used to quantify the number of signaling molecules being released from a vesicle and the number stored inside a vesicle, respectively. Fluorescence imaging is capable of providing spatial information related to exocytosis, including vesicle movements and exocytotic protein machinery, complementing the electrochemical techniques. The role of membrane lipids in regulating exocytosis can be studied using mass spectrometry imaging (MSI).

The main focus of the papers included in this thesis has been to investigate the alterations of exocytosis and membrane lipid composition in relation to cognition and the formation of plasticity. In paper I, the mechanism by which cocaine and methylphenidate (MPH) alter exocytosis as well as vesicular transmitter storage was studied in cells to understand the opposite effects of these two drugs on cognition. Paper II followed the release and storage of neurotransmitters during repetitive stimuli to understand activity-dependent plasticity. A quantitative comparison of SCA measurements between nanotip and disk electrodes was carried out in paper III to support the use of nanotip electrodes for both IVIEC and SCA. In paper IV, the effect of zinc deficiency on membrane lipid composition was examined in *Drosophila* brains using MSI and the results resemble the changes observed with cognitive-impairing drugs. As a follow-up study of paper II, paper V applied MSI to investigate the alterations of cellular membrane lipids induced by repetitive stimuli and suggested increased membrane curvature as the driving force to stabilize the exocytotic fusion pore. A technique using two nanotip electrodes to simultaneously perform SCA and IVIEC was developed in paper VI, enabling direct comparison between vesicular release and dynamically altered vesicular storage.

The combination of SCA, IVIEC, and MSI has provided useful insights into the mechanism and regulation of exocytosis. The concept of partial release, during which the fusion pore opens and closes to allow only a fraction of the vesicular transmitter storage to be released, has been supported. This is important as it suggests more pathways to regulate exocytosis. Vesicular release, storage, and fraction of release are all potential factors that can be manipulated to alter cellular communication, offering new possible targets for treating neurological diseases as well as understanding plasticity and memory formation.