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Visualization of Exocytosis and Endocytosis in Cultured Neurones Using TIRF Microscopy

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Membrane trafficking is essential for neuronal function: from growth of neurons and synapse formation to recycling of synaptic vesicles and receptors, questions concerning exocytosis and endocytosis are stimulating neurobiology research. In particular, glutamate receptors present in recycling endosomes are necessary for the expression of synaptic long term potentiation [1]. On the contrary, the endocytosis of glutamate receptors near synapses is necessary for the expression of long term depression [2]. The localization and the characteristics of exo- and endocytic events are thus crucial determinants of synapse function and plasticity.

To investigate these parameters, we have imaged cultured hippocampal rat neurons (7-22 days in vitro) transfected with receptors tagged with phluorin (pHl), a green fluorescent protein which is not fluorescent at pH 5.5 [3]. Receptors in acidic intracellular organelles are minimally fluorescent. Upon exocytosis, the fluorescence increases abruptly, revealing receptors on the plasma membrane. It is then possible to detect single exocytosis events [4]. In neurons transfected with tagged transferrin receptor (TfR-pHl), a receptor constitutively internalized and recycled at high rates, we detect 0.033 ± 0.007 events/μm^2/min ($n = 9$ neurons at 15 days in vitro). Similar exocytosis events are detected for other receptors and membrane proteins, such as the ionotropic glutamate receptor GluA1, the b2 adrenergic receptor, a G protein coupled receptor, or the SNARE protein VAMP2, albeit at a much lower rate. Close examination of exocytic events has revealed a large variety of behavior after exocytosis: in most cases, receptors diffuse quickly in the plasma membrane after exocytosis, but in about 25% of cases, receptors remain clustered. Using fast extracellular pH changes around the recorded cell [5], we show that these events are characterized by a short opening (median 2.5 s) followed by re-internalization. Finally, using two color imaging with markers of neuronal compartments, we have determined the endosomal origin of receptors undergoing this type of exocytosis. We will discuss the functional significance and the possible modulations of this phenomenon.

In addition, using a pH variation protocol developed previously [5], we have detected in neurons imaged with TIRF microscopy the formation of endocytic vesicles. We are currently characterizing the localization of endocytic zones relative to synapses. Finally, using patterned glass substrates, we investigate specialized neuronal compartments, hemisynapses, induced by the protein facing the responding neuron.

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