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Loading a calcium dye into frog nerve endings through the nerve stump: Calcium transient registration in the frog neuromuscular junction

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Abstract

© 2017 Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License. One of the most feasible methods of measuring presynaptic calcium levels in presynaptic nerve terminals is optical recording. It is based on using calcium-sensitive fluorescent dyes that change their emission intensity or wavelength depending on the concentration of free calcium in the cell. There are several methods used to stain cells with calcium dyes. Most common are the processes of loading the dyes through a micropipette or pre-incubating with the acetoxymethyl ester forms of the dyes. However, these methods are not quite applicable to neuromuscular junctions (NMJs) due to methodological issues that arise. In this article, we present a method for loading a calcium-sensitive dye through the frog nerve stump of the frog nerve into the nerve endings. Since entry of external calcium into nerve terminals and the subsequent binding to the calcium dye occur within the millisecond time-scale, it is necessary to use a fast imaging system to record these interactions. Here, we describe a protocol for recording the calcium transient with a fast CCD camera.

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Keywords

Calcium, Fluorescence calcium dye, Issue 125, Neurobiology, Neuromuscular junction, Neuroscience, Optical imaging, Presynaptic calcium transient

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