Doctoral College
Metabolic & Cardiovascular Disease

Surface traffic of L-type $Ca_V1.2$ Ca^{2+} channels in cultured hippocampal neurons

GUEST LECTURE by

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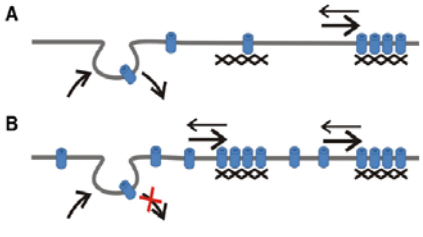
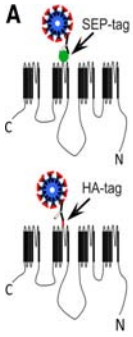
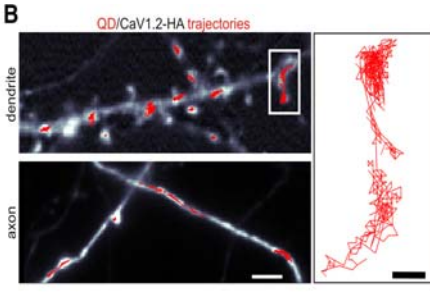
Abstract

In neurons L-type Ca^{2+} currents function in gene transcription and synaptic plasticity. The major neuronal L-type channels $Ca_V1.2$ are organized in clusters and form specific signaling complexes in dendrites and spines. Despite the physiological importance of L-type mediated Ca^{2+} currents, excessive Ca^{2+} influx leads to excitotoxicity and neurodegeneration. Thus, the tight control of $Ca_V1.2$ membrane levels and localization is essential to proper neuronal function. We used fluorescence microscopy techniques including fluorescence recovery after photobleaching (FRAP), live cell labeling protocols, and single particle tracking (SPT) to analyze the turnover and surface traffic of $Ca_V1.2$ in dendrites of mature hippocampal neurons. Pulse-chase surface labeling of extracellularly HA tagged $Ca_V1.2$ showed constant levels of membrane expressed channels within one hour, and dynamin-dependent block of endocytosis induced an increase in cluster density only after 30 minutes. Together these data suggest a turnover rate of $Ca_V1.2$s in clusters on the hour time scale. Surprisingly, FRAP analysis of $Ca_V1.2$ channels extracellularly tagged with super-ecliptic pHluorin revealed ~20% recovery within 2 minutes without reappearance of clusters, indicating the existence of a minor mobile population outside the clusters. Direct recording of the lateral $Ca_V1.2$ motions in the membrane using SPT showed that the majority of channels is highly confined in clusters, whereas a ~30% mobile pool exchanges between a confined and a diffusive state inside and outside of clusters, respectively. We conclude that an equilibrium of clustered and dynamic $Ca_V1.2$s maintains stable Ca^{2+} channel complexes involved in activity-dependent cell signaling. We propose that the channels mobile pool may provide capacity for short term adaptation in a developmental and activity dependent manner.



Model of the distribution and dynamics of $Ca_V1.2$ channels in dendrites of CNS neurons





Single particle tracking of $Ca_V1.2$ in the cell surface of dendrites and axons of hippocampal neurons

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Di Biase V, Tuluc P, Campiglio M, Obermair GJ, Heine M, Flucher BE