NAME: Qian Yang, qian.yang@vanderbilt.edu
PI: Colbran, Roger
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Activity-dependent clustering of L-type calcium channel complexes with Shank3 and CaMKII

Authors: Qian Yang, Lan Hu, Juliana C. Quay, Kirsty R. Erickson, Roger J. Colbran

Introduction: Learning and long-term memory require new mRNA transcription in neurons, which can be induced by many forms of excitation-transcription (E-T) coupling. Moreover, activity-dependent gene expression is disrupted in neurodevelopmental disorders, such as autism. One major E-T coupling mechanism is initiated by the activation of voltage-gated L-type Ca2+ channels (LTCCs) in the plasma membrane. In this pathway, increased phosphorylation of the nuclear CREB transcription factor can be induced by local Ca2+ increases within a nanodomain close to the channel, without requiring Ca2+ increases in the bulk cytosol or nucleus. Mechanisms underlying the formation of LTCC nanodomains are poorly defined. Previous studies in our lab found that LTCC-dependent E-T coupling requires direct binding of the LTCC α1 subunit N- and C-terminal domains (NTD and CTD, respectively) to Ca2+/calmodulin-dependent protein kinase II (CaMKII), a major neuronal signaling protein, and to Shank3, a scaffolding protein, respectively; as well as a novel CaMKII-Shank3 interaction[1,2]. We hypothesize that these three protein-protein interactions control LTCC clustering in the plasma membrane and the formation of LTCC nanodomains that are essential for efficient E-T coupling.

Methods: We transfected primary cultures of hippocampal neurons or HEK293 cells to express epitope-tagged CaV1.3 LTCC α1 subunits without or with Shank3a or CaMKIIα. Clustering of tagged α1 subunits was assessed by fluorescence microscopy (confocal or TIRF) or using a co-immunoprecipitation (co-IP) assay. Dynamic clustering of these proteins in the plasma membrane of live HEK293 cells was assessed by TIRF microscopy.

Results: We found that neuronal depolarization increases the size of CaV1.3 LTCC clusters in the dendrites of rat primary cultured hippocampal neurons. Our co-IP experiments indicate that Shank3a can assemble multimeric CaV1.3 LTCC complexes under basal conditions. Moreover, activated, but not inactive, CaMKII can assemble homo-multimeric CaV1.3 LTCC complexes, as well as heteromeric CaV1.3-CaV1.2 complexes in this co-IP assay. Using fluorescence microscopy, we found that the co-expression of Shank3a, but not CaMKII, enhances CaV1.3 cluster size in HEK293 cells under basal conditions. However, the addition of an LTCC agonist (BayK8644) to HEK293 cells co-expressing CaMKII increased the size of CaV1.3 clusters. Ongoing studies are examining dynamic changes in the size and number of LTCC clusters, as well as Shank3a and CaMKIIα colocalization, in live HEK293 cells.

Discussion: Our data indicate that clustering of CaV1.3 L-tyle calcium channels involves a complex interplay between basal effects of Shank3a and activity-dependent effects of CaMKIIα. We plan to investigate the impact of these novel molecular clustering mechanisms on local and global calcium signaling events following neuronal depolarization, as well as on E-T coupling.

References, if any: 1. Wang et al., 2017. J Biol Chem. 292(42) 17324, PMC5655510 2. Perfitt et al., 2020. J Neurosci. 40(10) 2000. PMC7055140

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