Activity-dependent clustering of L-type calcium channel complex with Shank3 and CaMKII

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Background

Learning and memory and gene transcription

- Learning and long-term memory requires new mRNA transcription in neurons, which can be induced by many forms of excitation–transcription (E-T) coupling

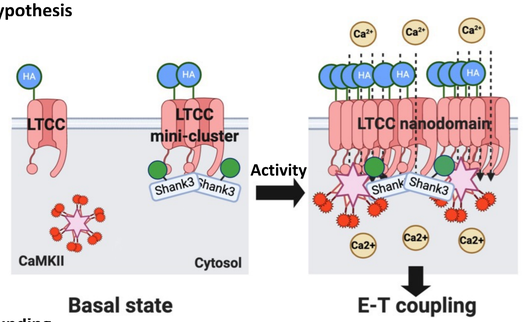
L-type Ca^{2+} channel (LTCC)-dependent E-T coupling

- E-T coupling can be initiated by local Ca^{2+} increases within a nanodomain close to voltage-gated LTCCs
- Ca^{2+} binds to its sensor, calmodulin (CaM), within the LTCC nanodomain, and Ca^{2+}/CaM then translocates to the nucleus in a complex with Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) to induce CREB phosphorylation
- This E-T coupling mechanism is essential for normal learning and memory (Cohen et al., 2018)
- Clustering of the major neuronal LTCC α1 subunits ($Ca_V1.2$, $Ca_V1.3$) may be critical for Ca^{2+} nanodomain formation and the initiation of E-T coupling

CaMKII and Shank3 are required for LTCC-dependent E-T coupling

- $Ca_V1.3$ α1 subunit N-terminal domains directly bind to activated dodecameric CaMKII holoenzymes, a major postsynaptic signaling protein
- $Ca_V1.3$ α1 subunit C-terminal domains directly bind to Shank3, a major postsynaptic scaffolding protein
- Shank3 directly bind to activated CaMKII holoenzymes
- Disruption of any one interaction between these three proteins interferes with E-T coupling (Wang et al., 2017. Perfitt et al., 2020).

Hypothesis



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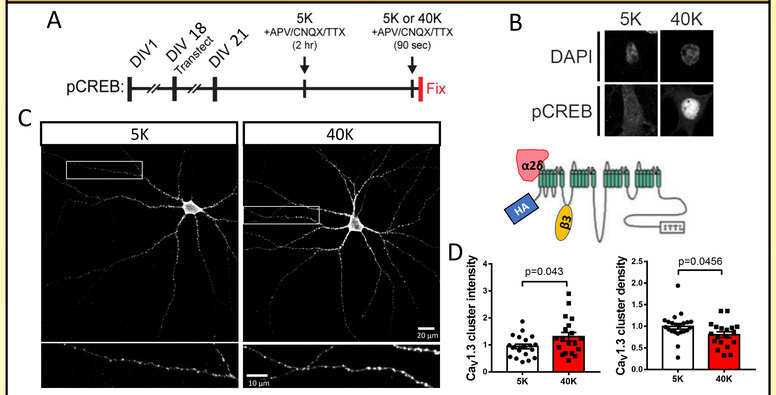
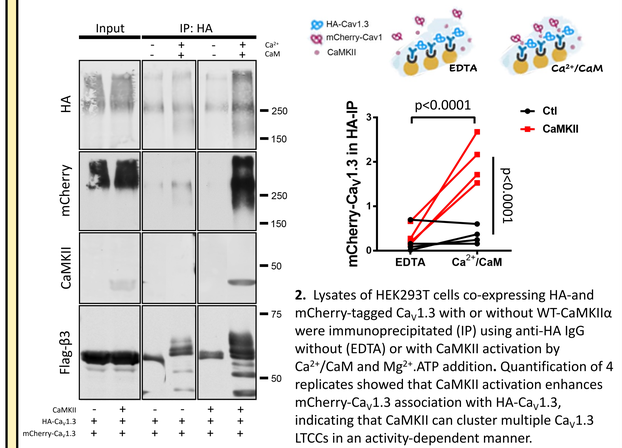
1. Activity-dependent regulation of dendritic $Ca_V1.3$ clustering in hippocampal neurons



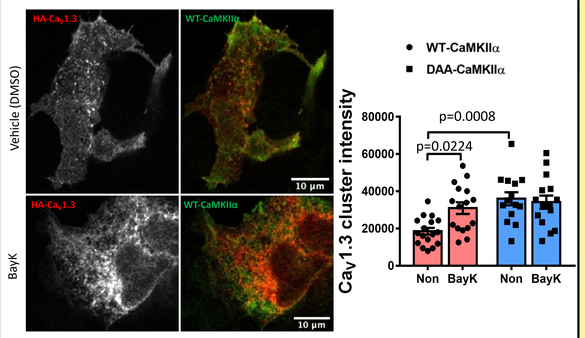
1. (A-B) Primary hippocampal neurons expressing HA-$Ca_V1.3$ were depolarized with 40 mM K^+ Tyrode's solution (40K) (or 5 mM K^+ control (5K)) using a protocol (schematic) that induces CREB phosphorylation (Wang et al., 2017). (C-D) Images of representative HA-antibody-stained whole cells with enlarged dendritic segments. Quantitation (D) showed that HA-$Ca_V1.3$ cluster intensity is increased, but the number of clusters is decreased by depolarization.

2. CaMKII clusters $Ca_V1.3$ L-type Ca^{2+} channels in a Ca^{2+}-dependent manner



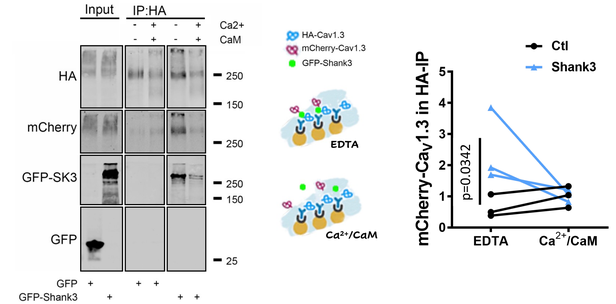
2. Lysates of HEK293T cells co-expressing HA-and mCherry-tagged $Ca_V1.3$ with or without WT-CaMKIIα were immunoprecipitated (IP) using anti-HA IgG without (EDTA) or with CaMKII activation by Ca^{2+}/CaM and Mg^{2+}.ATP addition. Quantification of 4 replicates showed that CaMKII activation enhances mCherry-$Ca_V1.3$ association with HA-$Ca_V1.3$, indicating that CaMKII can cluster multiple $Ca_V1.3$ LTCCs in an activity-dependent manner.

3. CaMKII promotes $Ca_V1.3$ clustering after Bay K8644 treatment in HEK293T cells



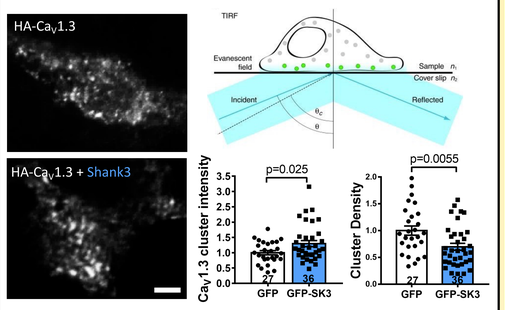
3. HEK293T cells co-expressing HA-$Ca_V1.3$ with WT- or constitutively active (DAA)-CaMKIIα were incubated for 20 min with LTCC agonist Bay K8644 (BayK) or vehicle (control) and stained with HA and CaMKIIα antibodies. BayK treatment increased $Ca_V1.3$ cluster intensity in the presence of WT-CaMKII compared to DMSO control, while DAA-CaMKII increased the cluster intensity under basal condition, and with no further increase after BayK treatment.

4. Basal clustering of $Ca_V1.3$ L-type Ca^{2+} channels by Shank3 is disrupted by Ca^{2+}/CaM



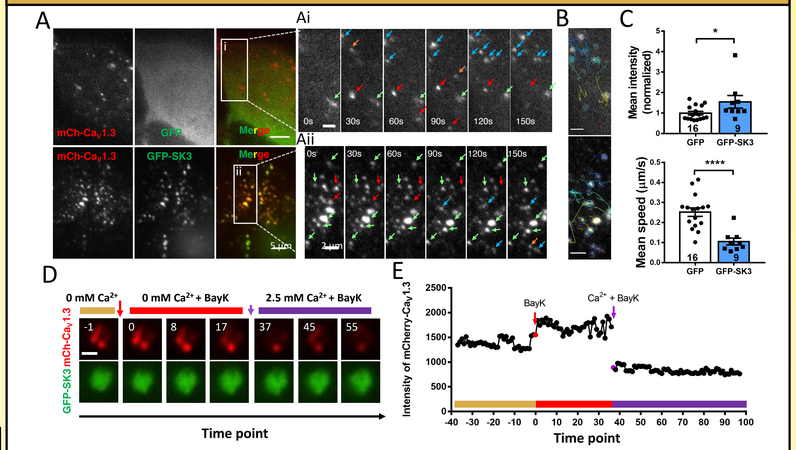
4. Lysates of HEK293T cells co-expressing HA-and mCherry-tagged $Ca_V1.3$ with or without GFP-Shank3 were immunoprecipitated (IP) using anti-HA IgG without (EDTA, basal condition) or with Ca^{2+}/CaM addition, simulating LTCC activation. Quantification of 3 replicates showed that Shank3 enhances mCherry-$Ca_V1.3$ association with HA-$Ca_V1.3$ under basal condition, but not following the addition of Ca^{2+}/CaM.

5. Shank3 enhances clustering of $Ca_V1.3$ in HEK293 cells under basal condition



5. HEK293T cells expressing HA-tagged $Ca_V1.3$ with GFP control or GFP-Shank3 were fixed, stained with HA antibody and imaged by TIRF microscopy. Representative images: scale:5 μm. $Ca_V1.3$ cluster intensity was increased, but cluster density was decreased by GFP-Shank3 compared to GFP control.

6. Dynamics changes of plasma membrane $Ca_V1.3$ LTCCs and Shank3 punctate in live HEK293T cells



6. (A) TIRF image of live HEK293T cells expressing mCherry-$Ca_V1.3$ with GFP or GFP-Shank3. (Ai/ii) Enlargement of highlighted areas at selected time points. Arrowheads highlight punctae with different properties: Green, stable. Red, disappear during session. Blue, appear during session. Orange, appear transiently. (B) Trajectories of $Ca_V1.3$ punctae are depicted on images of the last time point. Puncta intensity was increased, and puncta speed was decreased (C) in cells expressing GFP-Shank3 compared to GFP control. (D) Another live HEK293T cell expressing mCherry-$Ca_V1.3$ and GFP-Shank3 (not shown) with enlarged time-lapse images of a selected GFP/mCherry co-cluster. Data were initially collected in the absence of extracellular Ca^{2+}, and then following the successive addition of BayK and Ca^{2+}. (E) Reduced mCherry-$Ca_V1.3$ cluster intensity after Ca^{2+} addition may indicate that $Ca_V1.3$ LTCCs dissociate from Shank3 (Fig. 5) and are Internalized.

Summary

Conclusions

- Clustering of neuronal $Ca_V1.3$ L-tyle calcium channels may be modulated by membrane depolarization
- CaMKIIα activation enhances $Ca_V1.3$ LTCC clustering in HEK293T cells
- Shank3 enhances basal $Ca_V1.3$ LTCC clustering in HEK293T cells

Remaining questions

- How do CaMKII and Shank3 change the dynamics of $Ca_V1.3$ LTCC clustering?
- How does $Ca_V1.3$ clustering affect the formation of local Ca^{2+} nanodomains and E-T coupling?
- Does CaMKII enhance the co-clustering of $Ca_V1.3$ with other types of LTCCs?

Citations

- Cohen et al., 2018. Nature Comm, 9:2451
- Wang et al., 2017. J Biol Chem, 292(42):17324
- Perfitt et al, 2020. J Neurosci, 40(10):2000