Calcium Handling in Striatal Neurons in Huntington Disease

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Huntington Disease (HD) is a progressive neurodegenerative disorder caused by a CAG repeat expansion in the Huntingtin (HTT) gene. The striatum is preferentially degenerated, with additional atrophy of the cortex & other regions. Dysregulation of synaptic function & Ca2+ handling is common in many neurodegenerative disorders such as Alzheimer, Parkinson, & HD. One feature of HD is the dysregulation of Ca2+ at the endoplasmic reticulum (ER), whereby mHTT directly interacts with & sensitizes the IP3R1 to IP3, leading to ER Ca2+ store depletion & causing excessive Ca2+ store refilling. Activating Sigma-1 Receptor (S1R) normalizes these features.

Another important feature of HD is an increase in eNMDAR at cortical-overexpression of eNMDARs. The Bading Lab has shown that these features are closely mimics the established Ca2+ store leak in YAC128 neurons, can normalize nuclear calcium signalling. Dysregulation of synaptic function & Ca2+ handling is common in many neurodegenerative disorders such as Alzheimer, Parkinson, & HD. One feature of HD is the dysregulation of Ca2+ at the endoplasmic reticulum (ER), whereby mHTT directly interacts with & sensitizes the IP3R1 to IP3, leading to ER Ca2+ store depletion & causing excessive Ca2+ store refilling. Activating Sigma-1 Receptor (S1R) normalizes these features. Another important feature of HD is an increase in eNMDAR at cortical-overexpression of eNMDARs. The Bading Lab has shown that these features are closely mimics the established Ca2+ store leak in YAC128 neurons, can normalize nuclear calcium signalling. Dysregulation of synaptic function & Ca2+ handling is common in many neurodegenerative disorders such as Alzheimer, Parkinson, & HD. One feature of HD is the dysregulation of Ca2+ at the endoplasmic reticulum (ER), whereby mHTT directly interacts with & sensitizes the IP3R1 to IP3, leading to ER Ca2+ store depletion & causing excessive Ca2+ store refilling. Activating Sigma-1 Receptor (S1R) normalizes these features.

Methods

- Cortical & striatal tissues were dissected & plated from YAC128 or WT mouse embryos.
- Experiments were done at DIV 17-21.
- Transfection: Cortical-Striatal Co-Culture
- Imaging

Results

- Fig. 1: Cytosol-to-nuclear Ca2+ signal transmission is reduced in co-cultured YAC128 striatal neurons. A) Representative images of striatal neurons expressing both a nuclear localized GCaMP3 (nucleus) & a cytosolic localized jRCaMP1b (cytosol). B) Nuclear (green) & cytosolic (red) sample calcium traces. C) The amplitude ratio is the ratio of the amplitude of a nuclear event divided by its associated (synchronous) cytosolic event. D) Averages of nuclear (D) & cytosolic calcium amplitudes with an associated nuclear event (E). (WT, n=29; YAC128, n=29)

- Fig. 2: Cell-wide Ca2+ event amplitudes are decreased to varying degrees after Ryanodine, D-APV or Nifedipine. A) Representative image of a striatal neuron expressing jGCaMP7f. B) Calcium event amplitudes after 30 μM Ryanodine (Rya) treatment. Ryanodine was applied for 5 minutes after baseline recordings before imaging again (WT, n=22; YAC128, n=22). C) Calcium event amplitudes after 25 μM D-APV treatment (WT, n=21; YAC128, n=17). D) Calcium event amplitudes after 10 μM Nifedipine (Nif) treatment. p-values using two-way ANOVA (WT, n=11; YAC128, n=13). (BL = Baseline)

- Fig. 3: Cytosol-to-nuclear Ca2+ signal transmission is reduced after 30 μM Ryanodine (Rya) treatment. A) Nuclear (green) & cytosolic (red) sample calcium traces of striatal neurons expressing both a nuclear localized GCaMP3 (green) & a cytosolic localized jRCaMP1b (red). B) The amplitude ratio is the ratio of the amplitude of a nuclear event divided by its associated (synchronous) cytosolic event. C, D) Averages of nuclear (C) & cytosolic calcium amplitudes with an associated nuclear event (D). (WT, n=13; YAC128, n=10) (BL=Baseline)

- Fig. 4: Activin A secretion is decreased in YAC128 cortico-striatal co-cultures. Activin A ELISA was performed on the media of WT and YAC128 cortico-striatal co-cultures at DIV 4, 10, and 21. The Y axis shows Activin A fold increase relative to DIV4. (WT, n=5 batches; YAC128, n=5 batches)

- Fig. 5: Activin A overexpression differentially affects GluN2B surface expression in YAC128 striatal neurons. Representative images of striatal neurons stained for surface and internal GluN2B treated with either SHAM or Activin A. Stats of two-way ANOVA are in the figure. (WT, n=4 batches; YAC128, n=4 batches) (Act = Activin A)

Conclusion

- Our data suggests that:
  - Ca2+ signalling to the nucleus is impaired in YAC128.
  - The endoplasmic reticulum is an important player in cytosol-to-nuclear calcium signalling.
  - Activin A protein levels are decreased in YAC128 culture media.
  - Activin A overexpression & 3-PPP 1 μM treatment can normalize the overexpression of surface GluN2B.

- Future experiments will aim to link these mechanisms by assessing whether:
  - 3-PPP treatment can normalize nuclear Ca2+ signalling in YAC128 cultures.
  - 2. Ca2+ influx from opening Voltage-Gated Ca2+ channels.
  - 3. ER enhances Ca2+ signal due to CICR which facilitates Cytosol-to-Nuclear Signaling

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