Synaptic vesicle recycling is disrupted in a mouse model of Huntington's Disease

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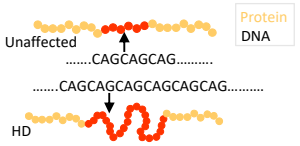
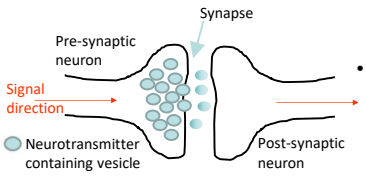
1. Huntington's Disease (HD) and neurotransmission

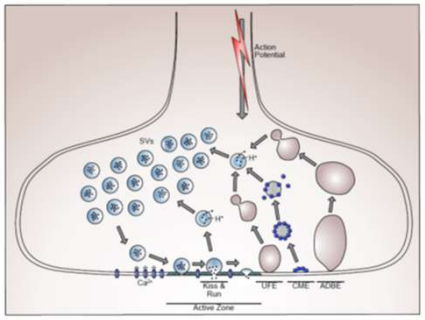
- HD is an inherited neurodegenerative disease of late onset.
- Symptoms include uncontrolled writhing movements and cognitive decline.
- Caused by expansion of a trinucleotide repeat in exon 1 of the *huntingtin* gene – affected individuals have >40 CAG repeats[1].





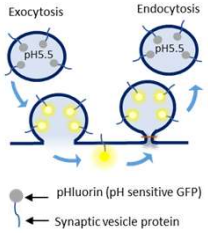
- Synaptic failure is a breakdown in neurotransmission - one neuron releases neurotransmitter molecules which activate the next at structures called synapses.

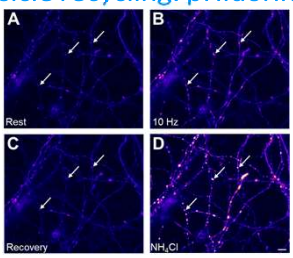
2. Presynaptic function - Synaptic vesicle (SV) recycling



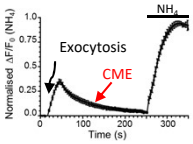
- Require localised vesicle recycling to maintain efficient neurotransmission[2]
- Action potentials elicit fusion of vesicles (EXOCYTOSIS) into the plasma membrane
- Membrane and protein cargo subsequently retrieved by ENDOCYTOSIS.
- Aberrant SV recycling can lead to synaptic failure and degeneration.

3. Monitoring synaptic vesicle recycling: pHluorins



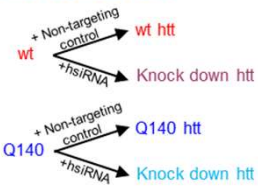


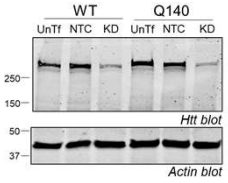
- Increase in fluorescence upon exocytosis and subsequent decrease in fluorescence reports SV retrieval (extent and kinetic information)[2].
- NH_4Cl pulse reveals total SV pool.

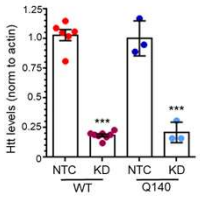


4. *Htt* expression in wt and Q140 neurons silenced

- Q140 model is knock-in with ~140 CAG repeats.
- *Mutant Htt* gene expressed at endogenous levels.
- Primary striatal neurons cultured from wt and Q140 mice.
- Hydrophobically modified siRNA silences wt and mhtt *expression*[3,4]. *(KD = htt knock down, NTC = non-targeting control)*

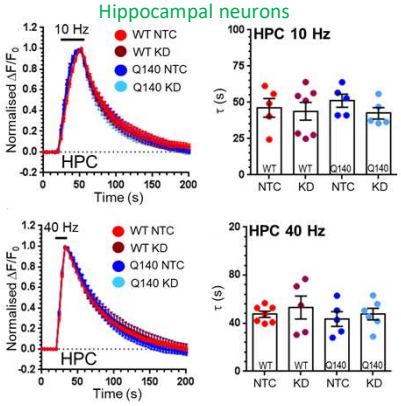


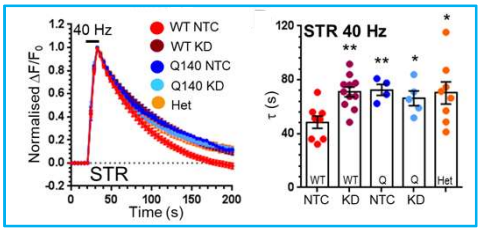




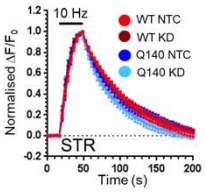
5. Q140 neurons display a striatum-specific, activity-dependent slowing of SV endocytosis

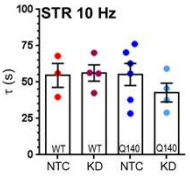
Hippocampal neurons





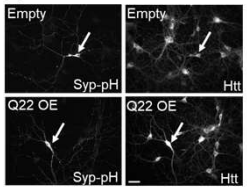
Striatal neurons

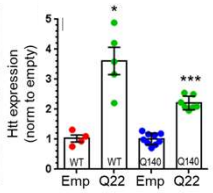


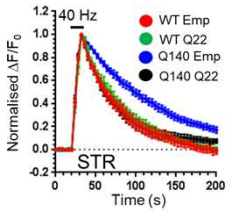


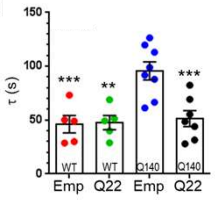
- SV endocytosis is unaltered in hippocampal neurons at low (10Hz) and high (40Hz) frequency stimulation[4].
- Q140 striatal neurons display slowing of endocytosis at high frequency activity[4].
- Knockdown of htt in wt cultures phenocopies Q140 slowing of endocytosis, suggesting loss of htt function underpins phenotype[4].
- Slowing of SV endocytosis also present in heterozygous Q140 cultures[4].

6. Over-expression of wt htt rescues slowing of endocytosis









- Htt with 22 CAG repeats was transfected in wt and Q140 neurons.
- Over-expression of htt in wt cultures did not alter SV recycling.
- Over-expression of htt in Q140 cultures rescues the slowing of SV endocytosis.

7. Summary

- Presynaptic dysfunction has been identified in a mouse model of HD.
- Specifically, SV endocytosis is slowed in an activity-dependent manner in striatal neurons.
- Defect due to loss of wt htt and can be rescued by re-introduction of wt htt.
- Heterozygous cultures also show the same phenotype, suggesting disease relevance.

References:
1. Saudou and Humbert Neuron 2016
2. Harper and Smillie J Neurochem 2021
3. Alterman *et al* Mol Ther Nucleic Acids 2015
4. McAdam *et al* Neurobio Dis. 2020