Investigating the role Huntingtin (HTT) plays on Rab19 and Rab3 motility in *Drosophila* larval axons

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Abstract

Huntington's disease (HD) is a neurodegenerative disease caused by poly-glutamine expansion in the huntingtin (HTT) protein. Previous work has shown that reduction in HTT causes axonal transport defects. While the vesicles that HTT affects during axonal transport are unknown, recent work has shown that reduction of *Drosophila* huntingtin (dHtt) dramatically disturbs the movement of Rab11, a recycling endosome protein. Using *in vivo* analysis, we explored how dHtt influenced the movement of fluorescently tagged Rab19 and Rab3 vesicles in *Drosophilia* larval axons. Rab19 is a recycling endosome while Rab3 is a synaptic vesicle protein. Using MATLAB particle tracking analysis, we examined the movement dynamics of Rab19 and Rab3 and found that both Rab19 and Rab3 display robust anterograde and retrograde movement in larval axons. Interestingly, reduction of dHtt dramatically reduced both anterograde and retrograde velocities of Rab3 vesicles, while only retrograde velocities of Rab19 vesicles were altered. Thus, our result indicates that HTT has a role in both Rab3 and Rab19 motility. Currently we are testing the hypothesis that Rab3 or Rab19 are on the same vesicle as Htt using dual color imaging.

What is Huntington's Disease?

Huntington's Disease (HD) is a genetic disease that degrades the brain. HD is caused by mutations in the HTT gene. There is currently no cure for HD

The Huntington protein (HTT)

Huntingtin (HTT) has been shown to function in cellular transport. HTT interacts directly with both kinesin and dynein-dynactin. Mutant HTT has also been shown to disrupt axonal transport (Gunawardena 2003). Therefore we will test the effects of HTT on the transport of Rab g-proteins.

Htt perturbs the motility of Rab3

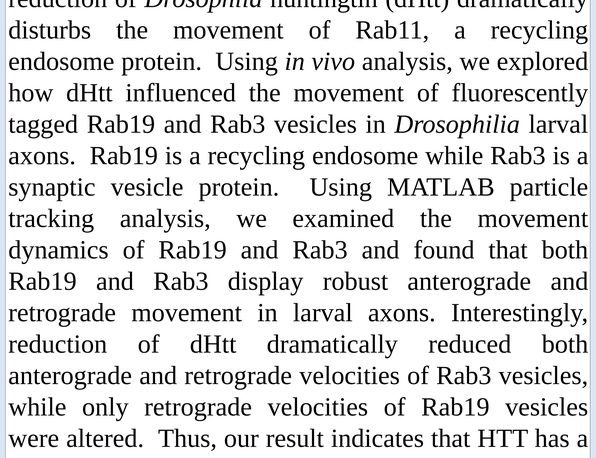


Figure 1: Rab3 is a synaptic vesicle g-protein. Larvae expressing Rab3 show robust vesicle movement, both anterogradely and retrogradely. With a 70% reduction of Htt in *Drosophila* larvae, there is less movement of Rab3 vesicles. There are blockages that inhibit movement of some vesicles.

Htt reduction reduces anterograde and retrograde velocities of Rab3

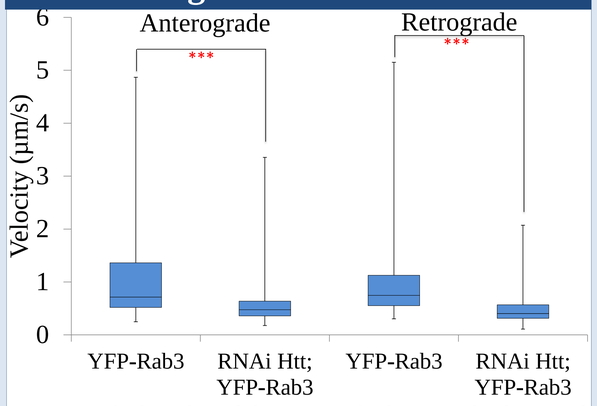


Figure 2: The box plots of duration weighted segmental velocities of YFP-Rab3 and RNAi htt;YFP-Rab3 (70% htt reduction) show that both anterograde velocity and retrograde velocity decrease significantly with decreased levels of Htt. This means that htt and Rab3 are interacting in some way (***p<0.001).

Rab3 vesicles pause more with Htt reduction

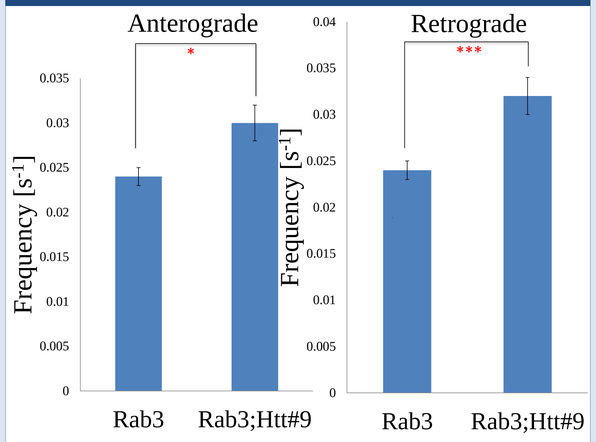


Figure 3: Bar graphs of the pause frequencies of YFP-Rab3 and RNAi Htt;YFP-Rab3 show that with a reduction of Htt, Rab3 vesicles pause more frequently, anterogradely and retrogradely (*p<0.015, ***p<0.001).

Rab3 vesicles pause longer with Htt reduction

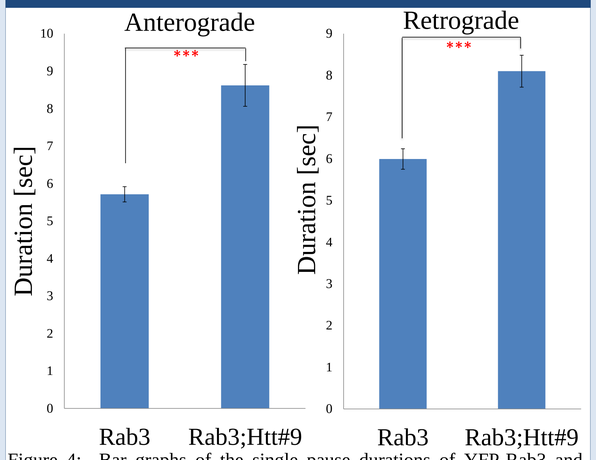


Figure 4: Bar graphs of the single pause durations of YFP-Rab3 and RNAi Htt;YFP-Rab3 show that with a reduction of Htt, Rab3 vesicles pause, both anterogradely and retrogradely, for a significantly longer period of time as they travel through the nerve (***p<0.001).

Htt perturbs retrograde movement of Rab19

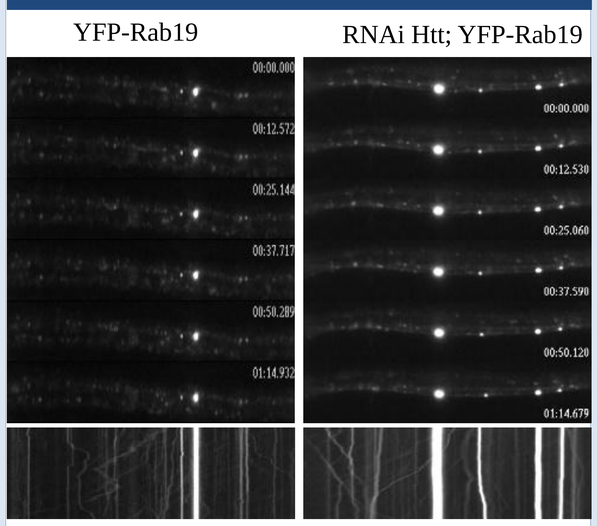


Figure 5: Rab19 is a recycling endosome g-protein. *Drosophila* larvae expressing YFP-Rab19 show robust bidirectional vesicle movement. RNAi Htt; YFP-Rab19 larvae have a 70% reduction of Htt. With the reduction there is less movement of Rab19 vesicles and more blockages prevalent.

Htt reduction reduces retrograde velocities of Rab19

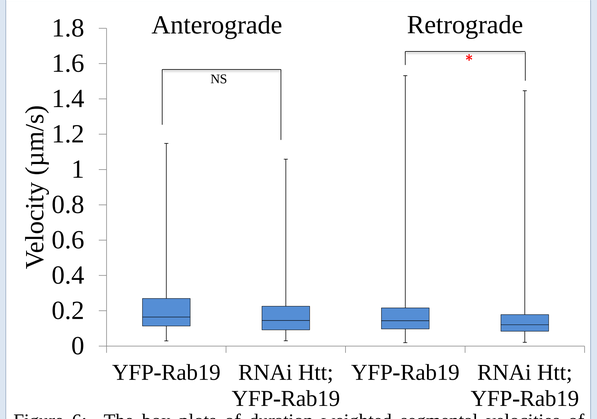


Figure 6: The box plots of duration weighted segmental velocities of YFP-Rab3 and RNAi htt;YFP-Rab3 (70% htt reduction) show only retrograde velocity decreases significantly with decreased levels of Htt. (*p<0.015, NS=not significant).

Htt reduction doesn't affect Rab19 vesicle pause frequencies

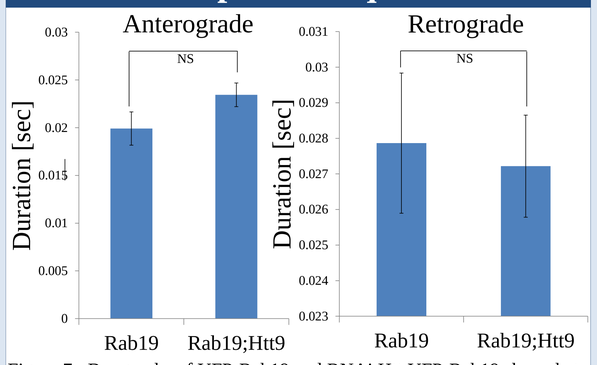


Figure 7: Bar graphs of YFP-Rab19 and RNAi Htt;YFP-Rab19 show that a reduction of Htt does not causes significant change in pause frequency in either anterograde or retrograde movement (NS=not significant).

Anterograde Rab19 vesicles pause for a shorter time with Htt reduction

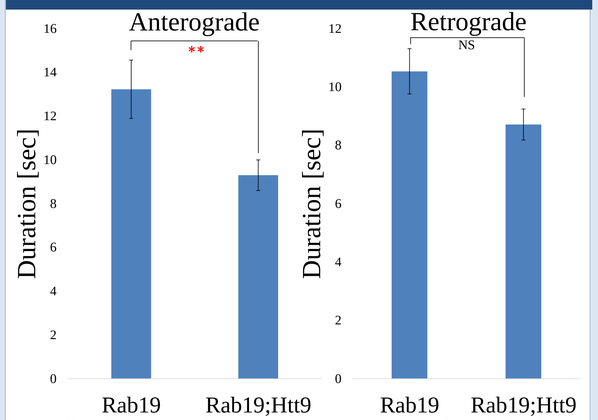
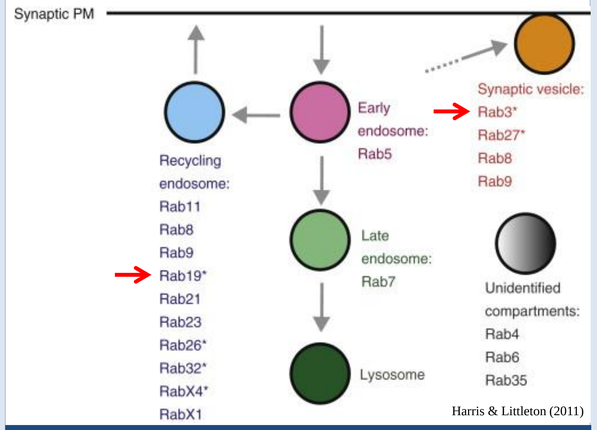


Figure 8: Bar graphs of the single pause durations of YFP-Rab19 and RNAi Htt;YFP-Rab19 show that with a reduction of Htt, only Rab19 vesicles traveling anterogradely pause for a significantly shorter period of time. There is no significant effect on retrograde single pause duration. (**p<0.01).

Conclusions

- Rab3 and Rab19 show bidirectional movement in *Drosophila* larvae
- HTT affects Rab3 and Rab19 motility
- HTT influences anterograde and retrograde velocities of Rab3
- HTT influences only retrograde velocity of Rab19
- HTT affects anterograde and retrograde Rab3 pause frequencies and pause durations
- HTT does not affect Rab19 pause frequencies
- HTT affects only anterograde pause duration in Rab19



Harris & Littleton (2011)

Future Directions

In the future we will work on determining if Rab3 or Rab19 are on the same vesicle as HTT. This will be made possible through the use of dual color imaging techniques of tagged proteins. We will also continue to track the movement of other Rab protein vesicles shown above to discover if other types of Rabs are also influenced by HTT.

Acknowledgements

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