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# Pruning the Garden of the Nervous System: Neurodegeneration in Drosophila

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## Introduction

- Neurodegeneration is caused by programmed cell death
- In general, mitochondrial structural alterations occur early in cell death
- Bruchpilot (German for crash pilot) is necessary for structural integrity and function of synaptic active zones in Drosophila
- RNAi provides a means to knockdown (decrease concentration of) specific proteins
- This study could have implications in finding treatments for human neurodegenerative diseases

## Main Questions

1. What effect will decreasing spectrin have on synapse formation?
2. Is there a relationship between Bruchpilot localization and synapse formation?
3. Do mitochondrial structural changes occur during the onset of neurodegeneration?

## Methods

- Dissection of *Drosophila* third instar larval nervous and segmental musculature
- Fixation for scanning (SEM) and transmission electron microscopy (TEM)
- Visual analysis of muscles and synapses by SEM and TEM
- Determine Bruchpilot localization at normal and mutant synapses by epifluorescence with a monoclonal antibody specific for Bruchpilot

## Results

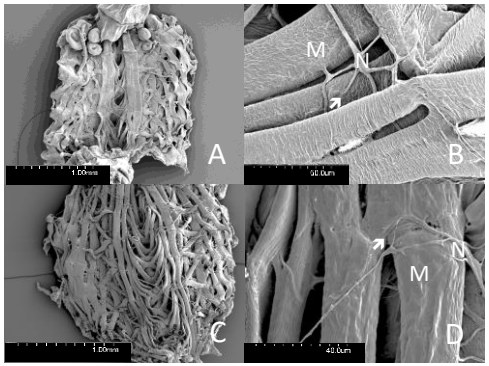


Figure 1. SEM images of *Drosophila*. A. Dissected WT larva. B. WT synapse of muscles 6 and 7. C. Dissected α-spectrin RNAi larva. D. α-spectrin knockdown synapse of muscles 6 and 7. Nerve (N), muscle (M), synapse (arrow).

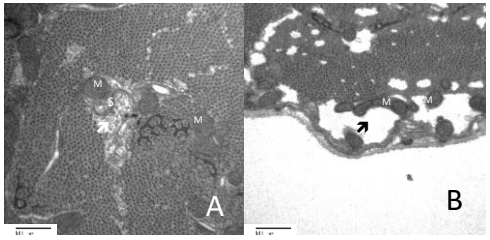


Figure 2. TEM images of *Drosophila* nerves and muscles. A. WT nerve. B. α-spectrin knockdown nerve. Synapse (S), mitochondria (M), muscle filament (arrow).

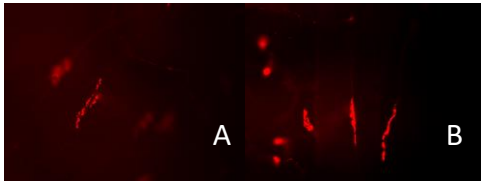


Figure 3. Images obtained by epifluorescence microscopy. A. Bruchpilot localization in WT synapses. B. Bruchpilot localization in α-spectrin knockdown synapses.

## Implications and Future Research

At low magnification with SEM (Figs. 1A,C), the WT and α-spectrin knockdown larvae are similar in overall pattern. Comparison of the synapses at high mag (Figs. 1B,D) shows that the knockdown of α-spectrin causes a separation of the nerve (N) from the muscle (M) at the synapse (arrow).

The WT TEM image (Fig. 2A) shows an apparently normal synapse (S) and normal mitochondria (M). In the α-spectrin knockdown (Fig. 2B) there appears to be degeneration of neuron filament organization (arrow). The inner membranes of the mitochondria in the mutant tissue appear to be disorganized compared to the mitochondria of the WT tissue, indicating that apoptosis is underway.

Bruchpilot localization in α-spectrin knockdown larvae is clearly less punctate (Figs. 3A and 3B), indicating that a decrease in α-spectrin does affect the localization pattern of Bruchpilot protein.

Future research should be focused more on which signaling pathway is affected by the knockdown of α-spectrin. Ankyrin (ank), another protein necessary for synapse stabilization, could be another avenue for neurodegeneration study. Preliminary research was performed on ank mutants, revealing flattened nerves with SEM visualization.

## Acknowledgements

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