Characterization of Axonal Transport in a Drosophila Neuronal Cell Culture System

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Abstract

The treatment of human neurodegenerative diseases has gained increased attention as their prevalence continuously is on the rise while effective treatment and knowledge of their origins remains unclear. The pathology of these diseases is characterized by a selective loss of neural tissue and the accumulation of proteins in plaques or inclusions. Despite the growing literature in the study of neurodegeneration, the underlying causes are largely unknown and a more comprehensive understanding of the disease pathways is needed for the development of effective treatment. Recently, defects in axonal transport have been implicated as an early event in neurodegeneration. This pathway is essential for highly polarized cells, like nerve cells, which rely heavily on efficient intracellular transport of vital protein cargoes for healthy function and structural integrity.

To determine how axonal transport defects are initiated, we have developed a Drosophila neuronal culture system to visualize, live the movement dynamics of several cargo proteins that are transported within the axon; fluorescent tagged synaptic vesicle proteins and mitochondria. Using this system we have generated several movies from day 1 and day 2 old cultures. For all of these cargos we observe dynamic bi-directional movement within neuronal projections. We track and analyze the movement behavior of these cargoes by generating kymographs from 15 second movies. In some instances we find axonal blockage assembly, disassembly and reassembly suggesting that these pathological blockages are more dynamic than previously thought using static and fixed immunolocalization experiments.

To further determine axonal defects we generated cultures from larvae containing motor protein mutations in Kinesin and Dynein. As expected, we find reduced motility and increased number of blockages in these cultures. These cultures also show a decrease in neuronal projection growth. Thus our method enables us to better understand the dynamic nature of axonal defects and to investigate how axonal defects are initiated in vivo.

Generating Primary Neuronal Cell Cultures For In Vivo Imaging

Grow larvae by crossing APPL-GAL4 virgin female flies with male flies containing the GFP tagged protein of interest.

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Dissect larvae, isolate the larval brain to generate primary neuronal cultures.

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Generate primary neuronal cultures.

↓

View cultures after 17 days at 100 x magnification with a Nikon TE2000-E inverted microscope.

↓

View cultures again after 41 days at 100 x magnification with a Nikon TE2000-E inverted microscope.

↓

At each time point, 10 isolated cells are imaged from each genotype. For each cell, four 30 second, 150 frame movies are taken at a 200ms exposure time.

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From each movie, the axon is identified and a kymograph is generated

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Vesicle dynamics are calculated.

GFP/YFP Tagged Vesicles Move Bi-directionally Within Neuronal Cultures

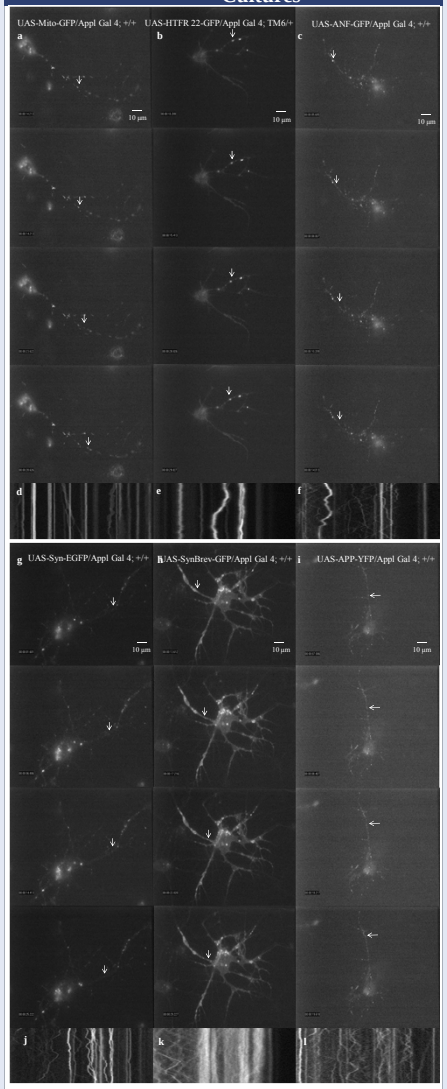

Figure 1: The movement dynamics of six tagged vesicles are observed in primary neuronal cultures: (a) Mitochondria, (b) Transferrin receptor, (c) ANF neuropeptide, (g) Synaptotagmin, (h) Synaptobrevin and (i) Amyloid Precursor Protein (APP). All vesicle classes show bidirectional movement. Four frames of a stream from each genotype are shown. Kymographs for all vesicle classes are also shown. Cultures were images at day 1 (17 hours) and again at day 2 (41 hours).

Neuronal Growth in Primary Neuronal Cultures

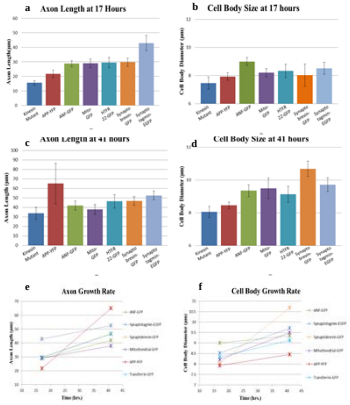

Figure 2: There is no significant difference in axon length at **(a)** 17 hours or **(c)** at 41 hours. No difference in cell body size is seen at **(b)** 17 hours or **(d)** at 41 hours. Axonal length was generated in Metamorph by tracing the axon from cell body to the terminus of the axon using the appropriate line tool. Neuronal cell body size was calculated in Metamorph using the calipers tool to measure the cell body diameter. N=10

Reduction of Kinesin Affects Neuronal Growth and Transport

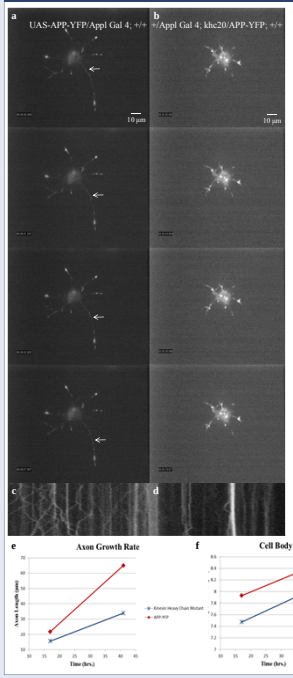

Figure 3: The reduction of anterograde motor, Kinesin, with APP-YFP leads to an impairment in axonal transport. **(a)** App-YFP movement is observed (arrow). **(b)** Kinesin mutants show axonal blockages compared to APP-YFP alone. The kymographs show **(d)** significantly less movement compared to **(c)** APP-YFP alone. Reduction of Kinesin also leads to a reduction of growth rates. The **(e)** kinesin mutant shows slower growth in axon length. **(f)** No significant difference is seen in cell body growth rates. N=10.

Axonal Blockages are Dynamic

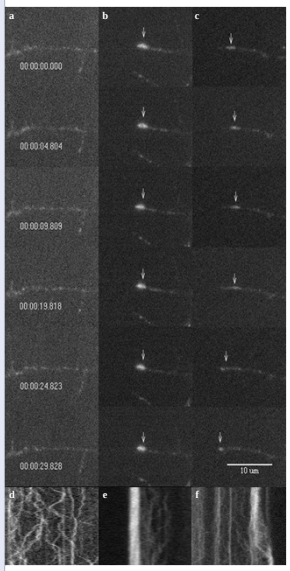

Figure 4: Axonal blockages are dynamics and can be categorized into different classes. **(a)** A neuronal cell expressing APP-YFP shows vesicle movement with no vesicle blockages. **(d)** The kymograph shows robust movement of vesicles. **(b)** A neuronal cell expressing APP-YFP shows a static blockage. Note that the block does not resolve (arrow). **(e)** The kymograph shows reduced movement. **(c)** A neuronal cell expressing APP-YFP with a dynamic blockage. Note that the block is moving and resolves over time (arrow). **(f)** The kymograph shows movement of the block.

Conclusions

- Bi directional movement is observed for all YFP/GFP tagged vesicles
- No significant differences are observed between different tagged vesicle proteins
- Reduction of Kinesin affects neuronal growth and axonal transport
- Axonal blockages are dynamic and can resolve over time.

Future Directions

- Does reduction of Dynein affect neuronal growth and axonAl transport in primary neuronal cultures?
- Are transport velocities observed in primary neuronal cultures similar to velocities observed in larval axons?
- what types of axonal blockages are detrimental to the cell?
- are resolving blocks benign?

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