Spatial and temporal characterization of normal axonal transport in primary neuronal cultures from Drosophila larvae

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Introduction

Neurodegenerative diseases are clinically observed to exhibit selective loss of neural tissue. These diseases are characterized for being **progressive** but the underlying molecular mechanisms of the pathology are unknown. One hypothesized mechanism is the **defective axonal transport** of cargoes vital for cell health and structure (Gunawardena et al., 2004). Correlations between disruption in axonal transport and some neurodegenerative diseases like Alzheimer's disease (Gunawardena et al., 2001), ALS (Tateno et al., 2009), Parkinson (Saha et al., 2004) and Huntington (Gunawardena et al, 2003) have been observed.

Axonal transport is essential for the movement of cargoes of molecules and organelles from the site of synthesis in neuronal cell body to their sites of utilization in the nerve termini. There are **two classes** of axonal transport:

- **Slow axonal transport:** movement of components of the cytoskeleton, mostly neurofilaments, tubulin and actin, at a rate of 0.1–20 mm/day.
- **Fast axonal transport:** movement of membrane bound proteins, mostly vesicles and mitochondria, at a rate roughly between 20 and 400 mm/day.

This transport can be divided in **two types** depending in which direction motor proteins travel along the microtubules:

- **Anterograde:** movement of cargoes from the cell body to the nerve termini.
- **Retrograde:** movement of cargoes from the nerve termini towards the cell body.

The two motor proteins responsible of accomplishing this transport are: **Kinesin** responsible of the anterograde transport and **Dynein** responsible of the retrograde. All of this is resumed in the Figure 1.
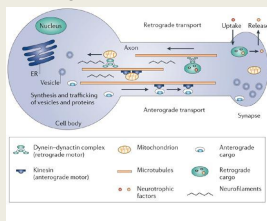

Figure 1: Normal axonal transport(Piera Pasinelli & Robert H. Brown, 2006)

Drosophila melanogaster is an organism model due to its simple and well understood genetics, the extend knowledge on its genome and its short life cycle. Furthermore, it has been proven to be a useful model for studying the role of axonal transport in disease. Drosophila melanogaster larvae are able to be dissected and segmental nerves imaged *Drosophila melanogaster* larvae are able to be dissected and segmental nerves used to observe the movement of fluorescently tagged cargoes (Fye, et al. 2010). Accumulations of molecules that do not move in the axons also called axonal blocks have been observed and related to defective transport.

Recently, Gunawardena's lab developed **a primary neuronal cell culture system** from Drosophila to study the spatial and temporal characterization of transport.

Methods

Figure 2: (A) Primary cell culture procedure (B) Culture and whole-mounted larvae immunohistochemistry protocol

Result 1: Neurons in primary cell culture are alive and electrophysiologically functional
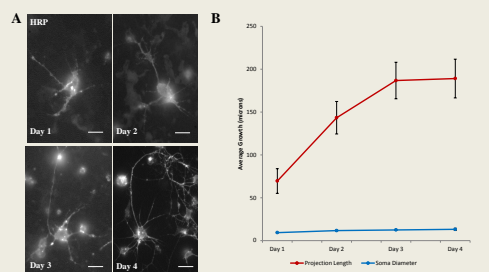

Figure 3: (A) Neurons in culture 1 (left, up) day and 2 (right, up), 3(left, down) and 4(right, down) days old stained with HRP (B) Quantification of growth during four days, blue line for the soma diameter and red line for the projection length, Bar = 20 microns. N = 10 cells.

Result 2: Neuronal markers show similar localization in both neurons in primary culture and whole mounted larvae
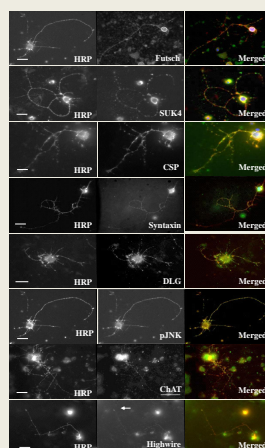

Figure 4: Two days old culture neuron stained with Futsch, SUK4, CSP, Syntaxin, DLG, pJNK, ChAT and Highwire. Growth cones are indicated with arrows. All presynaptic proteins show strong localization in the cell body. CSP, Suk4, ChAT, DLG and pJNK are strongly localize in the axonal projection while Futsch, Highwire and Syntaxin show strong localization in the growth as well. Bar = 20 microns; N = 10 cells.
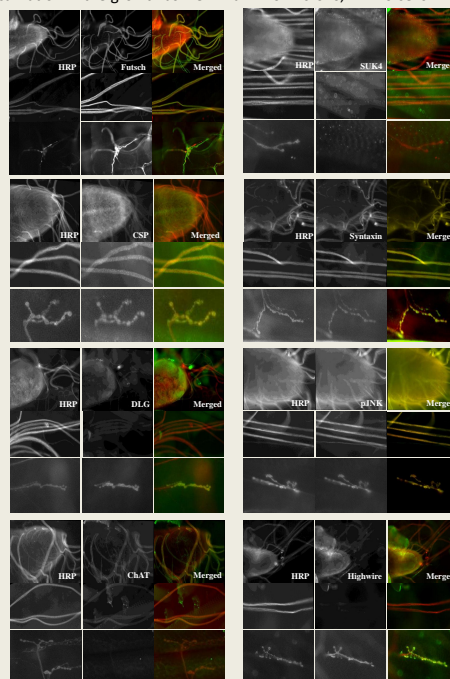

Figure 5: 3rd instar larvae stained with Futsch, SUK4, CSP, Syntaxin, DLG, pJNK, ChAT and Highwire. All presynaptic proteins are present in the ventral ganglion. CSP, Suk4, ChAT, DLG and pJNK are strongly localize in the segmental nerves while Futsch, Highwire and Syntaxin show also strong localization in the NMJ. N = 5 larvae.

Result 3: Bidirectional movement is observed in neurons of primary culture

Segmental Velocity Comparison
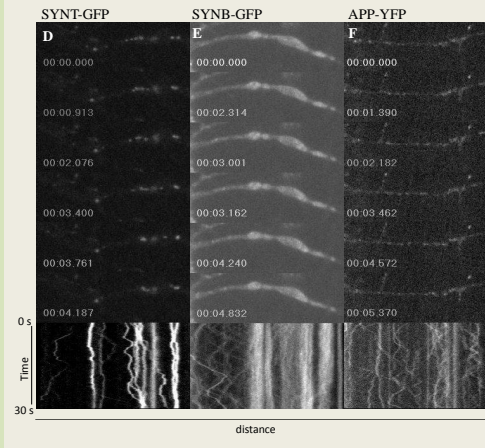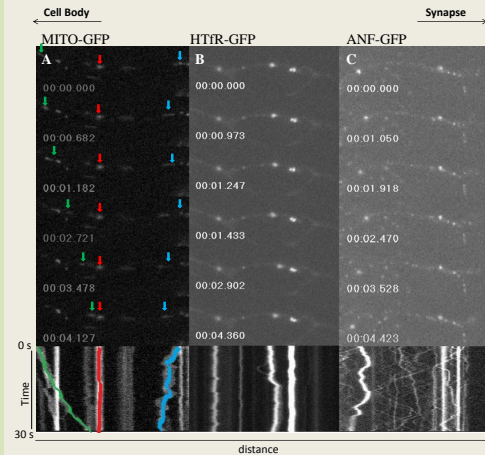
	Cell Culture		Whole Mount Larvae	
Cargo	Anterograde	Retrograde	Anterograde	Retrograde
APP-YFP	0.300 +/- 0.091	0.288 +/- 0.079	0.427 +/- 0.096	0.419 +/- 0.087
ANF-GFP	0.412 +/- 0.093	0.422 +/- 0.187	0.686 +/- 0.218	0.657 +/- 0.215
SYNT-GFP	0.327 +/- 0.102	0.313 +/- 0.221	N/A	N/A
SYNB-GFP	0.248 +/- 0.036	0.243 +/- 0.035	0.283 +/- 0.104	0.294 +/- 0.098
HTFR-GFP	0.473 +/- 0.117	0.416 +/- 0.072	0.363 +/- 0.121	0.365 +/- 0.161
MITO-GFP	0.387 +/- 0.060	0.356 +/- 0.063	0.479 +/- 0.094	0.443 +/- 0.084

Figure 6: To compare the movement between primary neurons and whole-mounted larvae six different cargoes were expressed (A) mitochondria (B) Transferrin Receptor (C) Atrial Natriuretic Factor (D) Synaptotagmin (E) Synaptobrevin (F) and Amyloid Precursor Protein in primary culture. Four consecutive 150-frame movies of cargo transport per cell or larvae were imaged at 100X with 0.126 micron/pixel spatial resolution and 5 frames/sec. temporal resolution. Bidirectional movement was observed. Red line indicates a stationary vesicle, the green one an anterograde transported one and the blue a reversing vesicle. **(Table):** segmental velocities in primary cell culture and whole mounted larvae; N=10

Conclusions

- Primary cultured neurons from third instar larvae develop normally and are electrophysiologically active.
- The cell body, projections and the growth cone of cultured neurons can be observed using neuronal markers.
- Neuronal markers localization is similar in cultured neurons and in the nervous system of larvae.
- Bi-directional movement is observed in cultured neurons.
- Expression of GFP/YFP tagged cargoes does not disrupt axonal transport.
- In vivo measurements of cargoes exhibit faster velocities compared to measured velocities in primary neuron culture.

Acknoslegments