Microtubule dynamics in Drosophila *models of neurodegeneration in search of a neuroprotective response*

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Abstract

Microtubules function as both structural components and as the tracks for transport in neurons. Studies have shown that defects in microtubule organization lead to neurodegenerative disease. Microtubules have their own organizational polarity, which can be assayed by tagging end-binding protein 1, EB1, with GFP. The majority of EB1 comets travel towards the cell body. The polarity can be assayed to identify a change in microtubule organization. In a previous study conducted by Chen et al, there was an upregulation in microtubule dynamics in *Drosophila* that served as models for poly-Q neurodegenerative diseases. The authors identified an increase in dendrite stability, which is thought to be microtubule based. When the poly-Q gene was expressed, dendrites did not prune back after being subjected to a pulsed-UV laser. The goal of this study is to identify whether other candidate genes implicated in various neurodegenerative diseases cause an upregulation in microtubules and an increase in stability. This study is critical to understanding the role of microtubule dynamics in a novel neuroprotective pathway.

Introduction

Neurons are a means of communication and drive many processes in the body. They are specialized cells that can send or receive signals from the brain. The three types of neurons are sensory, motor, and interneurons. The brain tells the body when to move, processes information, and receives information from both the body and the outside world. Without neurons the brain loses its method of communication with rest of the body, which has dire consequences.

In neurodegenerative disease the structure and function of neurons are progressively lost. These diseases include Alzheimer's, Parkinson's, ALS, and many others. Symptoms of these diseases may include, but are not limited to confusion, mental decline, muscle weakness, coordination problems, tremor, and involuntary movement[17-20]. Causes of disease and some key players have been identified [14, 16, 8], but there still remains many knowledge gaps to fill. Understanding the mechanism of disease may lead to improved treatments and patient outcomes.

The causes of neurodegenerative disease vary by disease, but many stem from copy number variation, and genetic mutations that can result in abnormal proteins. Spinocerebellar ataxias (SCA) and Huntington's disease are caused by trinucleotide expansion repeats. In a healthy individual, there are trinucleotide repeats, typically CAG repeats. Normally there are about 6 to 35 repeats in a person. In the disease state the trinucleotide repeat region is

expanded[14]. These trinucleotide expansion repeats may be due to defects in replication machinery, which slip when copying a repeat segment and then start over. The mechanism of expansion is not entirely clear, but studies support that expansion is due to this slippage during replication or defects in excision repair[12].

Other studies suggest that aberrant proteins may lead to neurodegenerative disease[16]. Prions are abnormal proteins that become infectious and can convert other proteins into the abnormal form. Alzheimer's, for example, is caused by the production of toxic alpha-beta (Aβ) peptide which accumulates and forms amyloid plaques. Aβ peptides are derived from an amyloid precursor protein (APP) that is cleaved by the enzymes BACE and γ-secretase. In addition to amyloid plaques, intracellular neurofibrillary tangles (iNFTs) are also present. INFTs are composed of tau protein which is a hyper-phosphorylated microtubule associated protein[14].

More recent studies have shown that defects in microtubule organization can lead to neurodegenerative disease[5]. Thus far, some key factors in the microtubule organization pathway have been identified. In the Rolls lab we continue to look for contributors of the microtubule organization pathway, and I used a microtubule assay in this experiment.

Figure 1: Microtubule organization is polymeric and

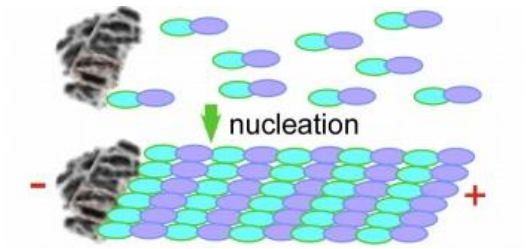
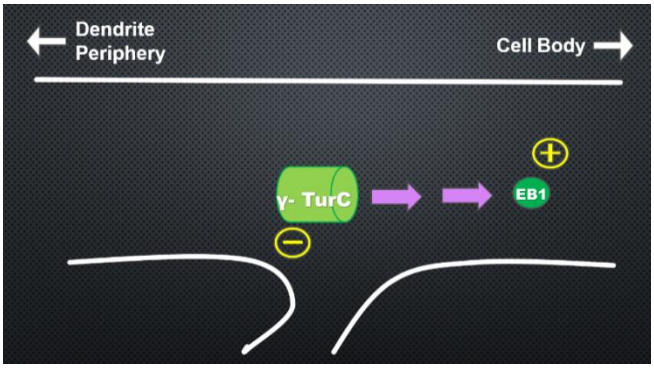


Figure 2: GFP tagged plus-ends travel through dendrite branch points



A previous study led by Li Chen in the Rolls Lab identified a microtubule response when neurons expressed poly-Q proteins that are associated with neurodegenerative disease. There was a statistically significant increase in microtubule dynamics. In addition to the microtubule response, dendrite stability increased. After a dendrotomy, the dendrites degenerate. In the study it was found that if they did an axotomy preceeding the dendrotomy, the dendrites would not prune back. It was hypothesized that a neuroprotective response was being activated due to the stress of the expressed transgenes[7].

The hypothesis is that the increased stability seen in the Li Chen study is due to the upregulation of microtubules. For our purposes, an increase in microtubule dynamics serves as an indication that there is a neuroprotective response being elicited in the dendrites. The question remains whether other triggers for neurodegeneration result in microtubule upregulation.

Results

Microtubule dynamics in dendrites with disease associated transgenes

The negative control had similar numbers of plus-end microtubules as other controls used in the lab previously. Since the plus-ends of microtubules are tagged with GFP, they can be counted when they pass the dendrite branch points. The total number of microtubules that passed at least one branch point were counted and divided by the length of the dendrite. This baseline is represented at 1 in **Figure 4**, and is labeled as Negative Control (Q27). The gene used in the Li Chen experiment was used in this experiment in order to obtain an idea of what the increase in microtubule dynamics looked like. The data collected from that repeated gene, Q78, served as a model of the microtubule response. Thus in **Figure 3** and **Figure 4** it is listed as Positive Control (Q78). The positive control did have a significant increase of 54.4%. The data was normalized to the negative control in microtubules per micron.

In an earlier study by Kleele et al., a microtubule response was found when mice overexpressed mutant Sod1[8]. Sod1 has been known to be associated with ALS. Sod1 is superoxide dismutase 1, which is an enzyme that works to eliminate toxins from the body. The enzyme plays a large role in eliminating superoxide radicals[2]. *Drosophila* males that expressed the human defective Sod gene1 were crossed with 221, Gal4, UAS EB1-GFP females to obtain the genotype, $Sod1/x\,;221, Gal4,\ UAS\ EB1-GFP/Sod1$. Microtubule comet tracking assays were taken for twenty-six animal organisms. The fold increase in microtubules per micron is 11% which is not statistically significant. Bonini et al. mention that *Drosophila* models with Sod1 may only serve as neurodegeneration models in the eye[11]. Other Sod1 forms will be tested including knockdowns of Sod1.

The defective gene labeled as MAPTau contains the human genes APP, BACE1, and MAPTau[1]. These three genes together are associated with Alzheimer's disease. As mentioned in the introduction APP is the amyloid precursor protein and BACE1 is a cleaving enzyme. MAPTau is a microtubule-associated protein that is hyperphosphorylated in Alzheimer's[3]. The microtubule change of 43.7% increase for MAPTau is not statistically significant when compared to Control Q27. However, it is trending upwards at a p-value of 0.08, and it may be statistically significant after more biological models are added.

Another control, yellow-white, was performed since it was crossed with the same tester line as MAPTau and Sod1. The tester line females used in these lines were 221, Gal4, UAS EB1-GFP. The average microtubules that passed branch points for the yellow-white larva was 0.445 MT/micron, which is higher than the average for MAPTau and Sod1. Due to the variation in this baseline data, the cross for yellow-white and data collection will be repeated.

Figure 3: Microtubule dynamics in *Drosophila* dendrites of overexpressed human disease proteins

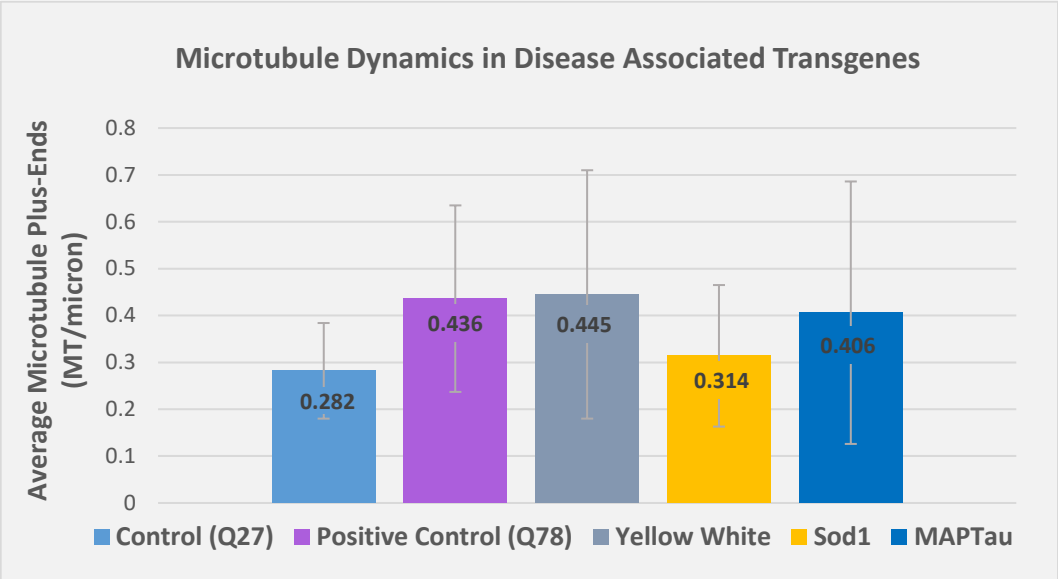
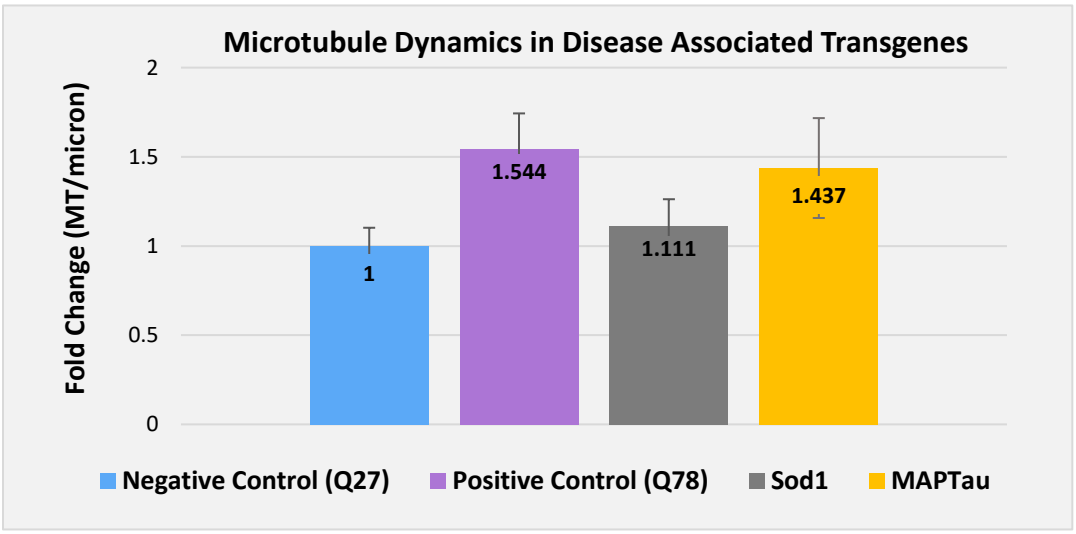


Figure 4: Percent change in microtubule dynamics for *Drosophila* with transgenes



Microtubule dynamics in dendrites with gene knockdown

There are several strategies to model neurodegeneration in flies: either by overexpressing human disease proteins or by knocking down *Drosophila* genes required for neuronal maintenance. The strategy used in this set of data is knockdown of genes. Dj-1alpha, also known as PARK7, is associated with Parkinson's disease. Dj-1 alpha is a positive regulator of androgen receptor-dependent transcription. It is also thought that it protects neurons against oxidative stress[3]. In this study dj-1 alpha had a statistically significant increase of 61.7% in microtubule dynamics (**Figure 6**). HtrA2 is also associated with Parkinson's disease. The HtrA2 gene

encodes a serine peptidase which is involved in apoptosis[2]. Thus, with the knockdown of HtrA2 there should be a decrease in the amount of apoptosis. In our study, when *Drosophila* expressed HtrA2 there was an increase of 78.3% in microtubule dynamics. Therefore, both the genes associated with Parkinson's disease elicit a microtubule response, which indicates a neuroprotective response (**Figure 5**).

Figure 5: Microtubule dynamics in *Drosophila* dendrites with gene knockdowns

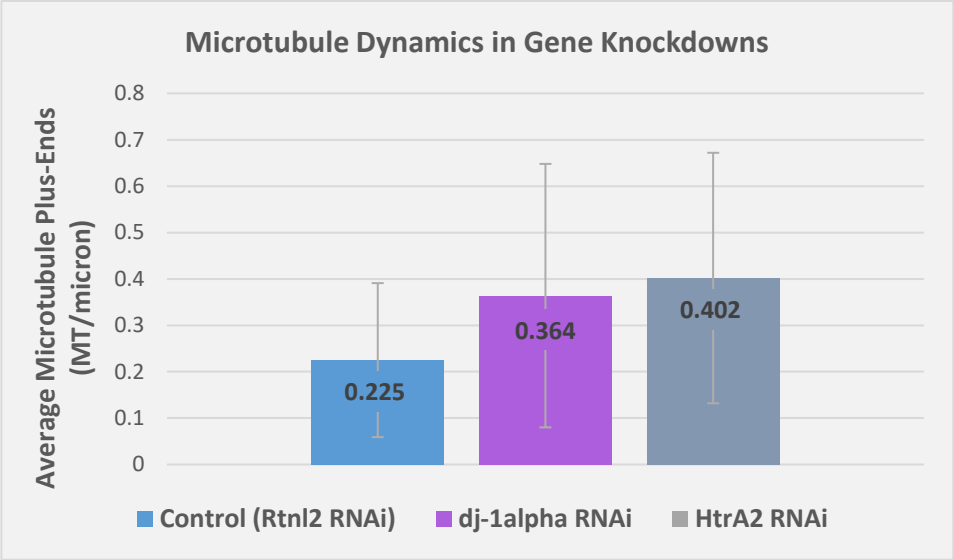
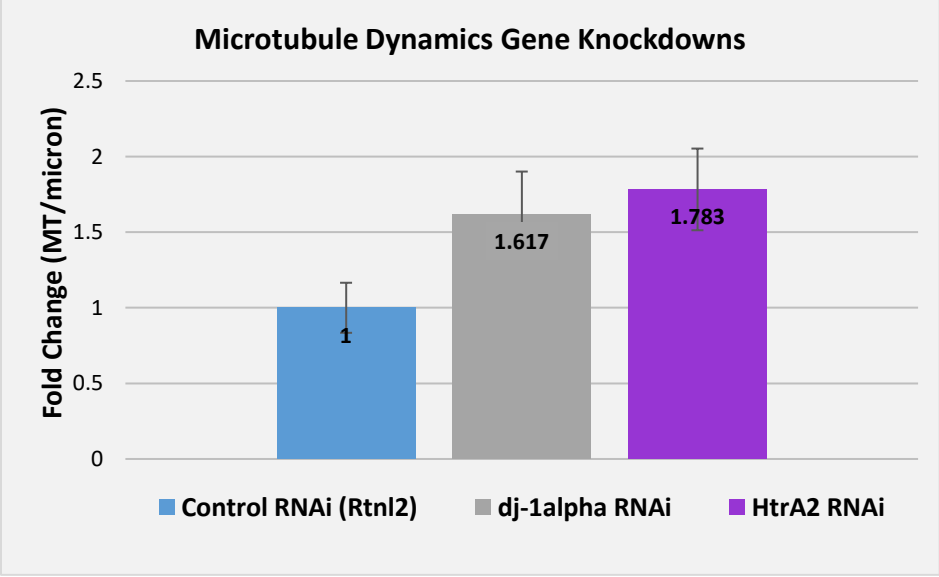


Figure 6: Percent change in microtubule dynamics for gene knockdowns



Discussion

The original hypothesis was that the neuroprotective response is a general characteristic among other neurodegenerative disease associated genes. With the current data that continues to be the hypothesis, since the majority of the genes tested had a statistically significant or almost statistically significant increase in microtubule dynamics. Despite data collected, we cannot say that there is also a neuroprotective response associated with these genes yet. We used the increase in microtubule dynamics as the primary indicator of the neuroprotective response, however we must still look for an increase in dendrite stability, through a lack of degeneration. Our next step is to subject dendrites to a pulsed-UV laser and look for degeneration. There are other genes associated with neurodegenerative disease that are on our list to test. After the screenings are done we will begin our research into learning how the neuron identifies that there is stress or injury present. Our goal is also to discover the neuroprotection pathway in order to use it for future treatment. If this pathway can protect temporarily protect neurons from neurodegeneration, the pathway could be turned on in disease patients in order to slow down disease progression.

Materials and Methods

Selection of cells for microtubule dynamics and injury

In *Drosophila* neurons are classified by how complex the structure is. To study microtubule dynamics we looked at the simplest neuron type, class I. In a cluster of neurons, the cell with its dendritic branches pointing towards the dorsal end of the larva is class I neuron, or ddaE cell. DdaE cells are sensory neurons and are commonly referred to as the comb dendrite.

Drosophila *stocks and crosses*

All *Drosophila* stocks were obtained through the Bloomington Stock Center. The tester line 221Gal4, UAS-EB1-GFP was used in order to express GFP in class I neurons and crossed with transgenic *Drosophila*. The transgenic *Drosophila* contained mutant forms of proteins which serve as models for various neurodegenerative diseases. *Drosophila* that had RNAi knockdown of proteins instead of mutant forms were crossed with the tester line Dicer 2; 221 Gal4, UAS EB1-GFP/TM6 in order to enhance the amount of knockdown. The Dicer2 female tester line was also crossed with Q27 and Q78 controls. The table below provides genes of the experimental lines and their descriptions provided by Bloomington. The table is organized by the disease that is modeled.

Gene Abbreviation	Chromosome Location	Tester Line	Progeny Genotype
Q27	3	Dicer2; UAS Gal4, 221, EB1-GFP/TM6	$\frac{Dicer\ 2;}{+}; \frac{UAS\ Gal4, 221, EB1-GFP}{Q27}$
Q78	2 or 3	Dicer2; UAS Gal4, 221, EB1-GFP/TM6	$\frac{Dicer\ 2;}{+}; \frac{UAS\ Gal4, 221, EB1-GFP}{Q78}$
Sod1	2;3	221 Gal4, UAS EB1-GFP	$\frac{Sod1}{+}; \frac{221\ Gal\ 4, UAS\ EB1-GFP}{Sod1}$
MAPTau	3	221 Gal4, UAS EB1-GFP	$\frac{221\ Gal\ 4, UAS\ EB1-GFP}{APP, BACE1, MAPT}$
Dj-1alpha RNAi	2	Dicer2; UAS Gal4, 221, EB1-GFP/TM6	$\frac{Dicer\ 2;}{dj-1alph}; \frac{UAS\ Gal4, 221, EB1-GFP}{+}$
HtrA2 RNAi	2	Dicer2; UAS Gal4, 221, EB1-GFP/TM6	$\frac{Dicer\ 2;}{HtrA2}; \frac{UAS\ Gal4, 221, EB1-GFP}{+}$
Rtnl2 RNAi	2	Dicer2; UAS Gal4, 221, EB1-GFP/TM6	$\frac{Dicer\ 2;}{rtnl2}; \frac{UAS\ Gal4, 221, EB1-GFP}{+}$

*Q78 labeled ambiguously. Labeled as stock number 8150 (8141) which have the mutation on different chromosomes.

Larva reproduction and growth

Stocks and crosses were kept at 25°C for optimal growth. Female virgins were selected from the tester line and crossed with males from the experimental line. The female *Drosophila* were given 24hrs to lay eggs and then the food cap was replaced. The larvae in the food cap were grown for three days so that they would reach the stage of third instar larvae. Changing the food cap daily ensures that all larvae within that cap are the same age.

Microtubule assay and quantification

A wide field Zeiss microscope (LSM510, Carl Zeiss, Oberkochen, Germany) was used to take videos of microtubule branch points. In all videos at least three branch points were in focus to ensure consistency and accuracy. In order to quantify the data collected, the amount of microtubule comets that passed through a branch point were counted and the direction (plus-end-in or plus-end-out) were noted. The total number of microtubules are divided by the length of the dendrite branch analyzed in order to find the total microtubules per micron. The experimental average MT/micron was compared against MT/micron of the control using a t-test.

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