Effects of Thrombospondin (TSP) on NMJ Structure and Locomotion of *Drosophila melanogaster*

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Introduction

The Neuromuscular Junction (NMJ) facilitates coordinated body movements. NMJ disorders, such as Myasthenia gravis, Isaac's Syndrome, and Botulism affect many people worldwide [1]. Individuals with NMJ disorders experience locomotor difficulties and muscle fatigue that can make daily life difficult [2]. Despite the effects on public health, little is known about the NMJ and the mechanisms that contribute to these disorders. Thrombospondin is a protein found at the NMJ, which may affect its formation and function.

- *Drosophila melanogaster* (fruit flies) are great model organisms because they have short life spans (Fig. 2), are low maintenance, and are estimated to share nearly 77% of disease-causing genes with humans [3,4] .
- Thrombospondins (TSPs) are proteins in the extracellular matrix that help with the formation of synapses [4].
- Humans have many TSP genes, making it difficult to determine the function of each one, while *Drosophila* have just one gene that codes for TSP.
- Different fly strains can be used to change the amount of TSP expressed within the neurons and muscles.
- Our lab's previous research has shown that strains with decreased expression of TSP had different NMJ structure.

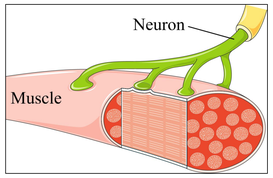


Figure 1. Neuromuscular Junction. The NMJ is the synapse, or connection, between a neuron and a muscle. There are proteins found here that help to form the synapse and hold it together, like thrombospondin (TSP). Modified from [5].

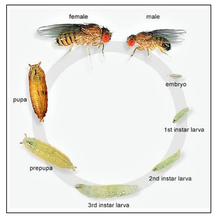
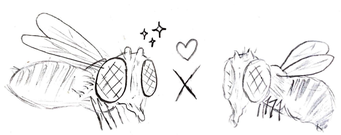


Figure 2. *Drosophila melanogaster* life cycle. Fruit flies typically live ~50 days in the lab and are typically grown at 25° C [4]. Modified from [7].

How does decreased expression of TSP in neurons or muscles change the development and behavior of the flies? We hypothesized that the strains with decreased TSP expression in either the neuron or muscle would have a different NMJ structure and/or pattern of movement than those with normal TSP expression.

Methods

- TSP expression in the muscle was selectively decreased using the Gal4-UAS system and a temperature-regulated repressor (Gal80ts), in which one strain contains an inactive RNAi sequence and the other contains a muscle driver sequence. These parent strains have normal TSP expression independently, but their offspring exhibit decreased TSP expression in specific tissues at 30°C (Fig. 3).
- Gal80ts is a temperature-dependent repressor that inhibits UAS-mediated gene expression but inactivates at higher temperatures, so flies raised in warmer conditions will exhibit stronger TSP knockdowns (Fig. 3).



w;elav-Gal4 **UAS-TSP RNAi**

tissue-specific knockdown

Figure 3. Cross scheme. Parental lines are phenotypically wild-type. One contains a genetic construct yielding tissue-specific expression of Gal4, a transcription factor, while the other has a corresponding UAS-TSP RNAi construct that will express in the presence of Gal4. The progeny of this cross will exhibit a selective knockdown of TSP within one tissue type depending on the Gal4 driver gene. The *w;elav* driver is expressed in neurons; *C600Dcr-2* is expressed in muscle. [8]

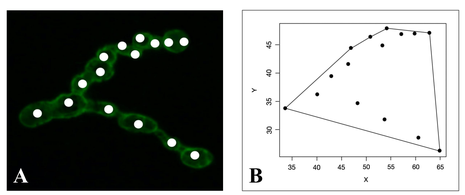


Figure 4. Convex Hull and Polygon Area analysis. A. Sample image of the confocal imaging of the synaptic portion of the NMJ. Each image was analyzed to assign coordinates to the location of each bouton. **B.** R was then used to analyze these coordinates using a convex hull to choose the outer points. These points were connected to create and find the area of an irregular polygon which is representative of the area of muscle covered by the neuron synapsing on it.

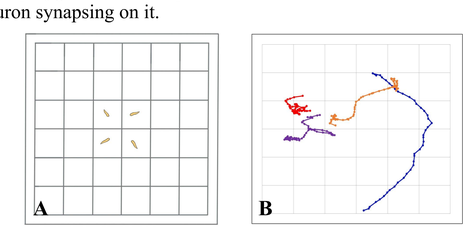


Figure 5. Larval locomotion. A. Depiction of the starting set up of larvae in the recording chamber for each video. **B.** LarvaTracker5000 software plotted the coordinates of their trajectories, which we used to analyze their movement.

Results

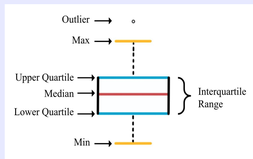


Figure 6. Box and whisker plots. Box and whisker plots display how a dataset is distributed. The box represents the majority of the dataset, which is the interquartile range (IQR). It's divided into the upper and lower quartiles (blue). The median (red) is shown with a solid line within the IQR. Whiskers connect the minimum and maximum values (yellow) to the box.

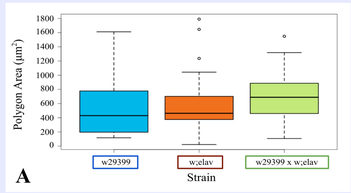
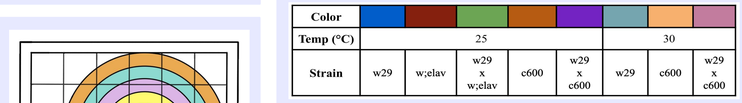


Figure 7. Polygon area is not significantly different when TSP expression is decreased. Data for each strain represents the distribution of areas found using the convex hull and polygon area analysis, all areas are reported in µm^2. This area corresponds to the estimated amount of muscle covered where the neuron synapses on the muscle. An outlier test was run on all datasets and significant outliers were removed [9]. A Shapiro-Wilk test was used to test for normality, which showed a non-normality result for some of the strains. This was followed by a Kruskal Wallis test, which showed no significant difference for any group of strains (p-values are reported for each). **A.** The polygon are of the TSP knockdown in neurons (*w29399 x w;elav*) is not significantly different from the parent strains. The Kruskal Wallis test reported a p-value of 0.09. All larvae were raised at 25°C. (n = 24, 30, 21) **B.** The polygon area of the partial TSP knockdown in muscle (*w29399 x C600Dcr-2*) is not significantly different from the parent strains. The Kruskal Wallis test reported a p-value of 0.783. All larvae were raised at 25°C. (n = 24, 27, 28) **C.** The polygon area of the TSP knockdown in muscle (*w29399 x C600Dcr-2*) is not significantly different from the parent strains. The Kruskal Wallis test reported a p-value of 0.158. All larvae were raised at 30°C. (n = 25, 17, 19)



Color								
Temp (°C)	25					30		
Strain	w29	w;elav	w29 x w;elav	c600	w29 x c600	w29	c600	w29 x c600

Figure 8. Fly strain color key. Each strain has a corresponding color that was used in a box around the name in their respective figures. Different colors were used to indicate when the same strain was grown at different temperatures. (w29 = w29399, c600 = C600Dcr-2)

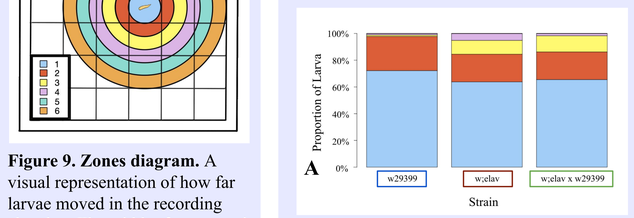


Figure 9. Zones diagram. A visual representation of how far larvae moved in the recording chamber. The width of one zone is approximately two larval body lengths, and the numbering of the zones corresponds to double the number of body lengths travelled from the origin. Each larva started in zone 1, and a maximum of 6 zones could be reached on the grid. Each color represents one zone, and these colors match those in figure 10.

Figure 10. TSP expression knockdown in the muscle results in differences in larval movement. A. *w29399* larvae reached zone 4 and the majority of larvae spent time in zones 1 and 2. *w;elav* larvae reached zone 4 and spent a greater proportion of time in zones 3 and 4 compared to *w29399*. *w;elav x w29399* larvae occupied zones 1 through 4 with similar proportions to the parent strain *w;elav*. These *w29399*, w;elav, and *w29399 x w;elav* larvae were raised at 25°C (n = 90, 58, 58). **B.** *w29399* larvae reached zone 4, and *C600Dcr-2* larvae reached zone 2. A similar proportion of larvae from these strains occupied zones 1 and 2. *w29399 x C600Dcr-2* larvae reached zone 3 and spent a greater proportion of their time there compared to the parental strains. These *w29399*, *C600Dcr-2*, and *w29399 x C600Dcr-2* larvae were raised at 25°C (n = 90, 38, 50). **C.** *w29399* larvae reached zone 3, and *C600Dcr-2* larvae reached zone 2. Larvae from both parental strains spent the majority of their time in zone 1. *w29399 x C600Dcr-2* larvae reached zone 4 and occupied more zones with a smaller proportion in each zone compared to the parental larvae. These *w29399*, *C600Dcr-2*, and *w29399 x C600Dcr-2* larvae were raised at 30°C (n = 55, 42, 50).

Conclusion & Future Directions

Conclusion:

There is no significant difference observed in polygon area when TSP expression is decreased in neurons or muscles. Significant alterations to zone occupancy were observed for larvae with TSP expression knockdown in the muscle, indicating that TSP plays a role in locomotive function at the post-synaptic terminal in the NMJ.

Future Directions:

- Validating efficacy of gene knockdowns through qPCR-based transcriptomic analysis.
- Developing an analytical approach to quantify larval locomotive behaviors and address discrepancies between genetic lines.
- Continue to investigate larval behavioral patterns of the combined muscular and neural knockdown line to further elucidate relationship between knockdowns.
- Further analysis of polygon area data, especially for NMJs with very small areas.

Acknowledgements

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