Characterization of Lis-1 Loss of Function at the Neuromuscular Junction of *Drosophila melanogaster* Larvae

Leticia Vargas[1,2], Jana Boerner[2] and Tanja Godenschwege[2]
[1]Honors Thesis Undergraduate Program
[2]Department of Biological Sciences, Charles E. Schmidt College of Science
Florida Atlantic University, Boca Raton, FL 33431

Abstract

Lissencephaly (smooth brain) is a brain malformation caused by mutations in the Lissencephaly gene Lis-1, causing incomplete neuronal migration. Our lab has investigated Lis-1 in *Drosophila melanogaster* using mutant phenotypes that parallel human Lissencephaly. In this study, we used *Drosophila* to determine if DLis-1 Loss of Function (LOF) impacts the synapse formation and axonal growth at larval neuromuscular junctions (NMJ's). Given that it is thought that DLis-1 protein is affecting Nrg localization, we further investigate the affects of DLis-1 LOF on Nrg. **We hypothesize that the DLis-1 LOF larvae will have an altered NMJ morphology with an accumulation of Nrg within the synapse that overall causes an interruption in synaptic function, such as crawling behavior.** This study will allow for a better understanding of the peripheral synapse of the *Drosophila* larvae affected by DLis-1 in correlation with Nrg.

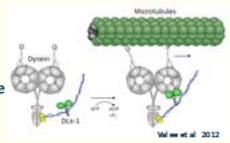
Background

DLis-1 and Neuroglian

- Neuroglian (Nrg) is a CAM, or cell adhesion molecule, that is important in axonal outgrowth and migration.
- DLis-1 has been found to affect Nrg localization [1].
- Our lab has shown that loss of DLis-1 function causes accumulation of Nrg at the Giant Fiber synapse [unpublished data].

Retrograde Transport

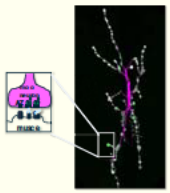
- Lissencephaly interacts with the motor complex of microtubules by binding with Dynein [2].
- Dynein bound with DLis-1 have a greater affinity to bind with the microtubules, thus enhancing retrograde transport [2].



Vallee et al 2012

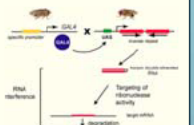
Larval Neuromuscular Junction

- Synaptic connection of the motor neuron to the muscle fiber.
- Model system to study synapse formation and function.



Genetic Crosses

- Used the UAS-Gal4 system.
- Used RNAi lines to down regulate the protein of interest in motor neurons.

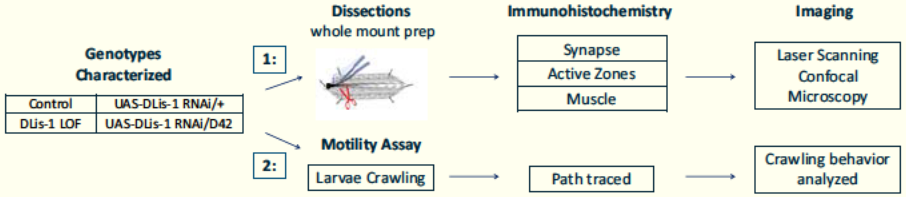


Antoniewski Christophe "Annual Report of Drosophila Genetics and Epigenetics for Year 2008." Inst. Jacques Pasteur 2008

Objectives

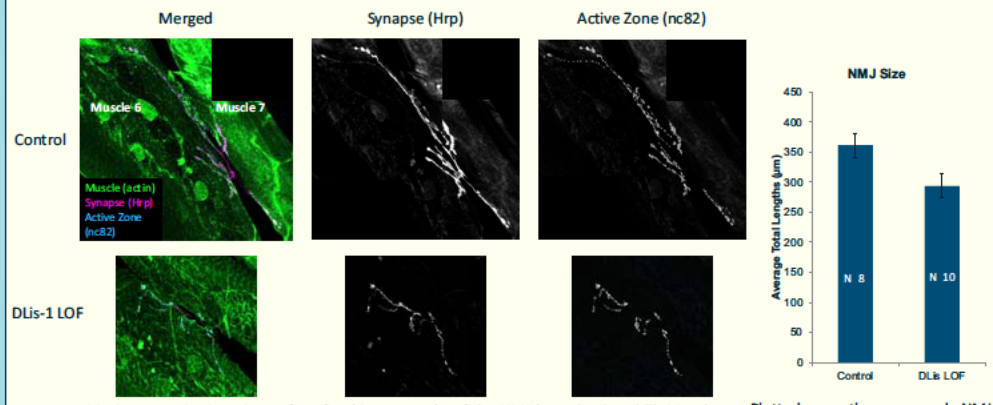
To quantify the morphology of the NMJ in DLis-1 LOF. To observe if the larvae NMJ are hindering or enhancing the motor behavior via crawling assays.

Experimental Approach



Preliminary Results

NMJ Morphology

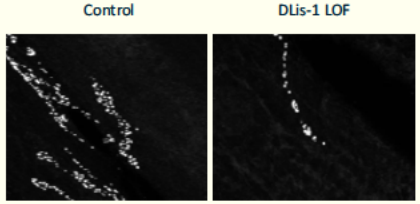


Maximum projection views of confocal image stacks of the NMJ (muscles 6 and 7). Muscles, neurons, and active zones were labeled with fluorescent antibodies. Staining of Hrp was used to measure NMJ length.

Plotted are the averaged NMJ lengths of the wildtype and the DLis-1 LOF with standard error.

• DLis-1 LOF NMJ are smaller and show less branching.

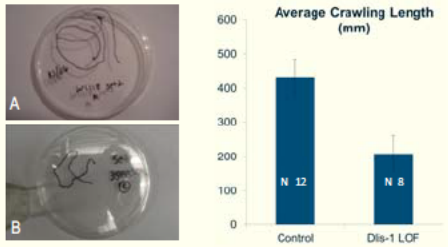
Active Zone Abundance



Maximum projection view of high resolution confocal image stacks from NMJs labeled with nc82, an active zone marker.

• NMJs with reduced levels of DLis-1 seem to have fewer active zones.

Crawling behavior



Example of crawling path traces of control (A) and DList-1 LOF (B). Plotted are the average lengths measurement.

• Motility seems to be reduced in DLis-1 LOF larvae.

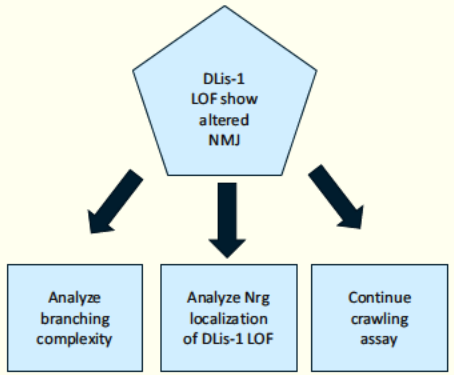
Conclusions

- DLis-1 loss of function is required for synapse growth in neuromuscular junction.
- DLis-1 LOF larvae exhibit shortened NMJ with less branching.
- Preliminary data suggest crawling is inhibited in DLis-1 LOF.

Discussion

Preliminary data shows that down regulating DLis-1 will cause a morphological change in the NMJ structure. The shortened NMJ with fewer branches also show to have fewer active zones, thus causing a reduced crawling behavior. Others have shown that DLis-1 is important in retrograde transport by interacting with the dynein motor. Loss of DLis-1 function in our model might disrupt signaling from the synapse to the central nervous system and inhibit synapse formation during development. This is possibly due to the unbound Dynein not transporting proteins back to the soma and therefore inhibiting cell signaling.

Future Work



References

1. Williams, M. J. (2009). *The Drosophila cell adhesion molecule Neuroglian regulates Lissencephaly-1 localisation in circulation immunosurveillance cells*. BMC Immunol, 10, 17.
2. Schnapp, B. J., Reese, T.S. (1989). *Dynein is the motor for retrograde axonal transport of organelles*.ProcNatlAcadSci. USA. 1548-1552
3. Valle, R.B., Mckenney, R.J., Ori-Mckenney, K.M. (2012) *Multiple Modes of Cytoplasmic Dynein Regulation*. Nature Cell Biology 14, 224-230.
4. Boerner, J., Godenschwege, T. (2010). *Application for the Drosophila ventral nerve cord standard in neuronal circuit reconstruction and in-depth analysis of mutant morphology*. J. Neurogenetics 10, 1067-7063.
5. Godenschwege, T., Kristiansen, L., Uthaman, S., Hortsch, M., Murphey, R. (2006). *A conserved role for drosophila neuroglian and human l1-cam in central-synapse formation*. Current biology 16, 12-23.

Acknowledgements

Dr. Evelyn Frazier, Dr. John Nambu, Dr. Ken Dawson-Scully, Julie Freund, Ramon Garcia-Areas, Stephania Libreros

Support by the National Institute for Child Health and Human Development (R01HD05072) is gratefully acknowledged.