Shot Protein Knockdown Reduces Surface Coverage in Drosophila Sensory Neurons

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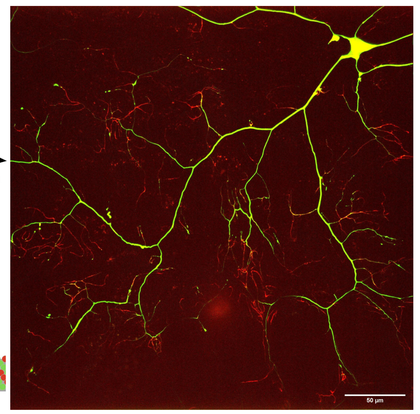
Abstract

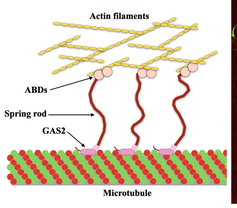
Neurons rely on tight coordination between the microtubule and actin cytoskeleton components to extend and maintain axons and dendrites over long distances. Disruption of this coordination is increasingly implicated in neurodegenerative disease, where a destabilized cytoskeleton contributes to axonal and dendritic degeneration and a loss of intracellular transport [1]. We use *Drosophila* C4da sensory neurons to investigate how the cross-linker protein Short stop (Shot) mediates physical binding between microtubules and the actin mesh. By knocking down Shot expression with RNA interference [2] and comparing microtubule and actin organization, as well as overall neuron morphology, against wild-type controls, we test the hypothesis that Shot helps anchor the actin mesh in place, enabling new dendrites to branch. We use live imaging of *Drosophila* neurons and systematic morphological analysis to quantify the relationship between Shot and structural phenotype. We found that the neurons of Shot-knockdown flies have a higher mesh size yet equal total branch length in comparison to wild-type controls, revealing that reduced Shot inhibits the cell's ability to nucleate new dendritic branches. These findings will clarify how loss of cytoskeletal cross-linkers drives neuronal structural breakdown, with broader relevance to cytoskeletal dysfunction in neurodegenerative diseases.

Background

Dendrites cover mesh of the larva surface, acting as actuators "sensing" mechanical pressure



Shot cross-linking actin on lattice



Microtubule: Rigid cytoskeletal tracks that support intracellular cargo transport [3]

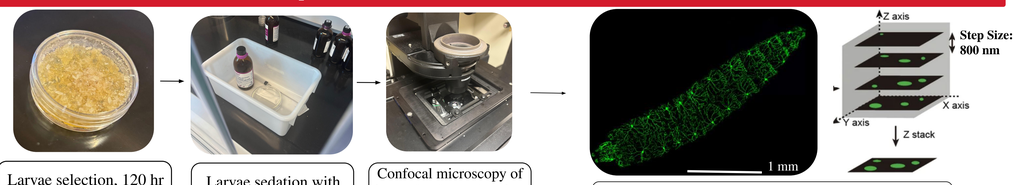
Actin: Dense, membrane-associated mesh that generates the local forces needed to shape dendritic branches [3]

The actin and microtubule systems have very different roles, so maintaining a properly organized neuron requires them to be coordinated. This is thought to rely on cross-linker proteins that physically bridge the two networks together. However, the exact proteins that perform this role, and how the loss of that physical link affects neuron structure, is not well understood.

Drosophila C4da sensory neurons offer a powerful model for studying this coordination in the context of neurodegenerative disease: their dendritic arbors are largely flat and easily imaged in live tissue, and up to 75% of human disease genes are conserved in flies [4]. Our project focuses on Shot, a candidate cross-linker that binds actin at one end and microtubules at the other. Our research asks whether reducing Shot expression is sufficient to disrupt this coordination and alter dendrite morphology.

Experimental Methods

1) Imaging



Larvae selection, 120 hr after egg-laying → Larvae sedation with isoflurane → Confocal microscopy of larvae in vivo, using thin z-stacks → Result: Series of images at varying focal depths

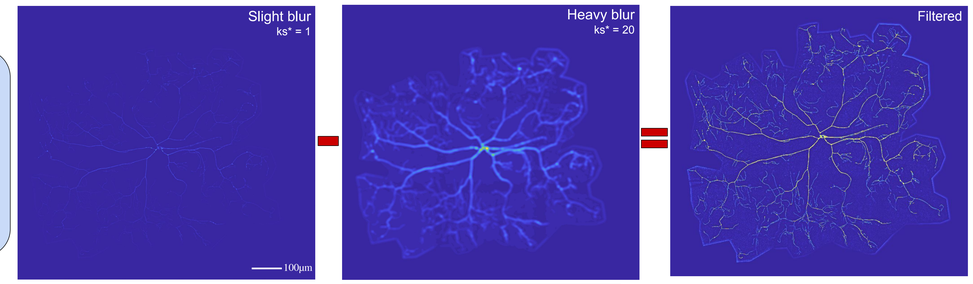
2) Processing

Maximum projection: Condense the 3D images onto a 2D plane using a looping scheme on MatLab → **Stitching**: Use normalized cross-correlation to align overlapping neuron images and merge them into one → **Cropping**: Highlight only on the subtree of importance on Fiji Image Editor

Result: Specified 2D image of one neuron that can be compared (control vs. shot group) along different modes of analysis

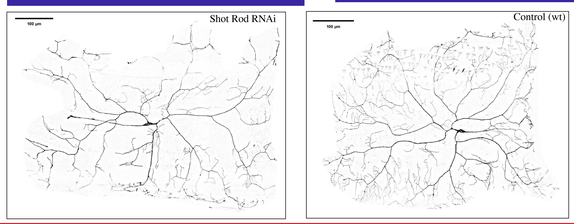
3) Analysis

Use **Gaussian filtering** to clean images and calculate…
→ Mesh size
→ Total branch length
→ Neuron density map



* ks = Gaussian kernel size

Use **Napari neuron tracing** to calculate…
→ Leaf number
→ Branch order
→ Branch length
→ Detecting actin and microtubule intensity

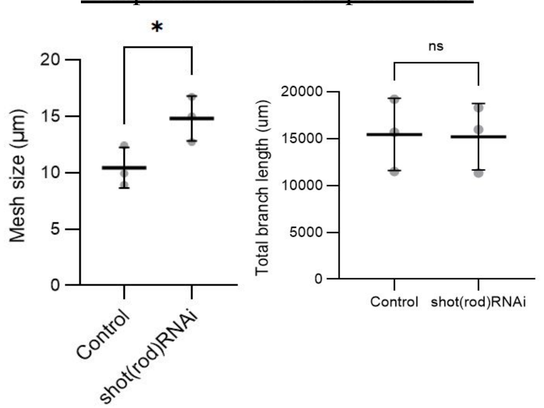


Results

	Mesh Size* (µm)		Total Branch Length (µm)	
Samples	Control Group	Shot Rod RNAi Group	Control Group	Shot Rod RNAi Group
1	8.9	12.1	15,660	18,305
2	12.4	15.0	19,183	15,957
3	9.9	16.7	11,478	11,320

* Mesh size characterizes a neuron's ability to cover an area (box length where a randomly placed box has a 50% chance of overlapping a dendrite). Smaller mesh sizes indicate efficacious coverage, while larger mesh sizes indicate structural vacancies [5].

Independent Two-Sample t-Tests



There is a statistically significant difference between the mesh sizes of the control group and shot RNAi group. Total branch length is conserved.

Conclusion and Future Steps

Both groups have very similar total branch lengths, yet the distribution of their dendrites is different.

- Control neurons densely cover larvae surface.
- Shot RNAi neurons, while conserving total dendritic length, cover larvae surface more sparsely. This may lead to reduced capability to detect mechanical threat.
- Absence of shot hinders nucleation of dendritic branches, verifying our hypothesis that Shot *does* enable neurocellular growth by crosslinking the cytoskeletal components

Next Steps

Building on the labelled data, we need to quantify the interaction between microtubule (MT) and actin through Shot cross-linkers using fluorescent markers. Several methods of computational analysis include determining the intensity of actin and microtubule and the neuron density map. We will also continue studying cross-linkers by experimenting with different levels of removing Shot (using MARCM) and analyzing other protein cross-linkers (e.g. clip, rho).

References

[1] Milatovic, D., Montine, T. J., Zaja-Milatovic, S., Madison, J. L., Bowman, A. B., & Aschner, M. (2010). Morphometric Analysis in Neurodegenerative Disorders. Current Protocols in Toxicology, 46(1). https://doi.org/10.1002/0471140856.tx1216s46.

[2] Heigwer, F., Port, F., & Boutros, M. (2018). RNA interference (RNAI) screening in Drosophila. Genetics, 208(3), 853–874. https://doi.org/10.1534/genetics.117.300077.

[3] Jan, Yuh-Nung, and Lily Yeh Jan. "Branching Out: Mechanisms of Dendritic Arborization." *Nature Reviews Neuroscience*, vol. 11, no. 5, 2010, pp. 316–328, doi.org/10.1038/nrn2836.

[4] Prokop, A. (n.d.). A rough guide to Drosophila mating schemes. http://halfonlab.ccr.buffalo.edu/courses/BCH512/Roote+Prokop-SupplMat-1v2.2.pdf Wikipedia contributors.

[5] Ganguly, Sujoy, et al. "Morphology of Fly Larval Class IV Dendrites Accords with a Random Branching and Contact Based Branch Deletion Model." *arXiv*, 16 Apr. 2018, arxiv.org/abs/1611.05918.

Acknowledgements

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