Developing a non-invasive intravital imaging strategy for analysis of pre-synaptic microtubule dynamics at the *Drosophila* neuromuscular junction

V.T. Chou[1], H. Lai[2], J.B. Long[1], M. Arnes[1], K. Obbad[1], L.A.G. Lucas[2], S. Alworth[3], J.S.J. Lee[2], D. Van Vactor[1]

Abstract

Microtubules (MTs) play critical roles in the development of synapses, but their regulation in this context is only beginning to be understood at the molecular level. In particular, little is understood about how the dynamic growth and shrinkage of MTs contribute to pre-synaptic growth and morphogenesis. MT dynamics have been analyzed in *Drosophila* tissue culture and sensory dendrites. However, due to the less favorable signal/noise ratio and higher background inherent to many *in vivo* and *ex vivo* tissue preparations, studies of pre-synaptic MT dynamics at the fly neuromuscular junction (NMJ) have been comparatively limited. To address this, we have optimized a novel software platform, Aivia (formerly SVCell), for automated and unbiased *in vivo* 3D particle detection and tracking that effectively addresses the challenges of the *Drosophila* NMJ. We performed non-invasive intravital imaging of EB1-GFP labeled MTs both in sensory dendrites and at the NMJ. We quantified multiple MT dynamics parameters in both cell types, finding striking differences in MT behaviors between sensory dendrites and at the NMJ. We furthermore analyzed sensory dendrite MT dynamics using multiple drivers of EB1-GFP expression and identified significant differences in distinct driver backgrounds. Thus, we demonstrate a novel strategy for intravital imaging and multiparametric analysis of pre-synaptic MT dynamics at the NMJ of intact animals, which we have used to identify distinct MT behaviors in different cell types and driver backgrounds. Our future studies will combine our imaging and software platform with traditional Drosophila genetics to dissect signaling networks and cytoskeletal effectors that orchestrate MT dynamics in the development of the synapse.

Methods

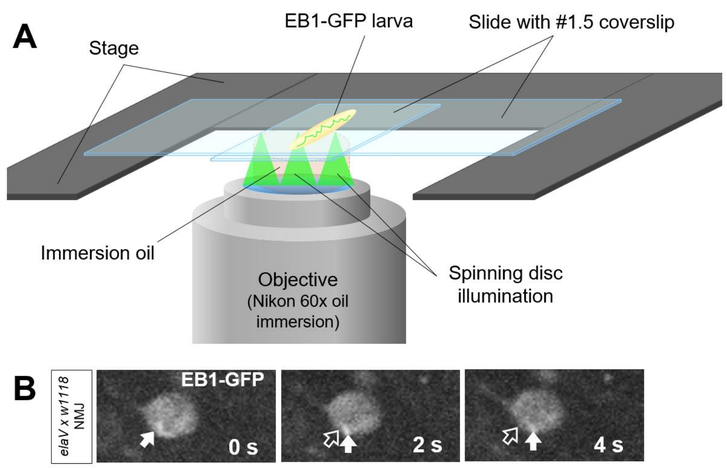

Figure 1. Live imaging strategy. **(A)** Third-instar larvae were anesthetized and imaged with spinning disc confocal illumination on an inverted Nikon Ti microscope at 2 second intervals. **(B)** EB1 tracking in wild-type *(elaV^{C155}-Gal4 x w1118)* NMJ. At t=0s, the solid arrow indicates the start position of the EB1-GFP puncta; at t=2 and 4 s, solid arrows indicate the current position, while clear arrows indicate the start position.

Table 1. Description of Tracking Parameters

Tracking Parameter	Description
Mean Comet Velocity	average of the detected track velocity (scalar) over the lifetime of the track
Max Comet Velocity	highest value of track velocity (scalar) detected over the lifetime of the track
Straight Line Velocity	growth length divided by the growth lifetime
Mean Acceleration	average of the rate of change of detected track velocity (scalar) over the lifetime of the track
Sinuosity	growth length divided by path length
Mean Square Displacement	sum of the particle displacement squared at all time points divided by growth lifetime
Growth Length	straight line distance between the starting frame position and ending frame position of the track
Growth Lifetime	total length (in time) of the detected track
Path Length	total distance traveled by the track

Acknowledgements We thank the Nikon Imaging Center at Harvard Medical School for technical help. This work was supported by the NIMH of (1R43MH100780-01).

Author Affiliations [1]Department of Cell Biology and Program in Neuroscience, Harvard Medical School, Boston, MA [2]DRVision Technologies LLC, Bellevue, WA [3]AcuraStem Inc., Los Angeles, CA

Results

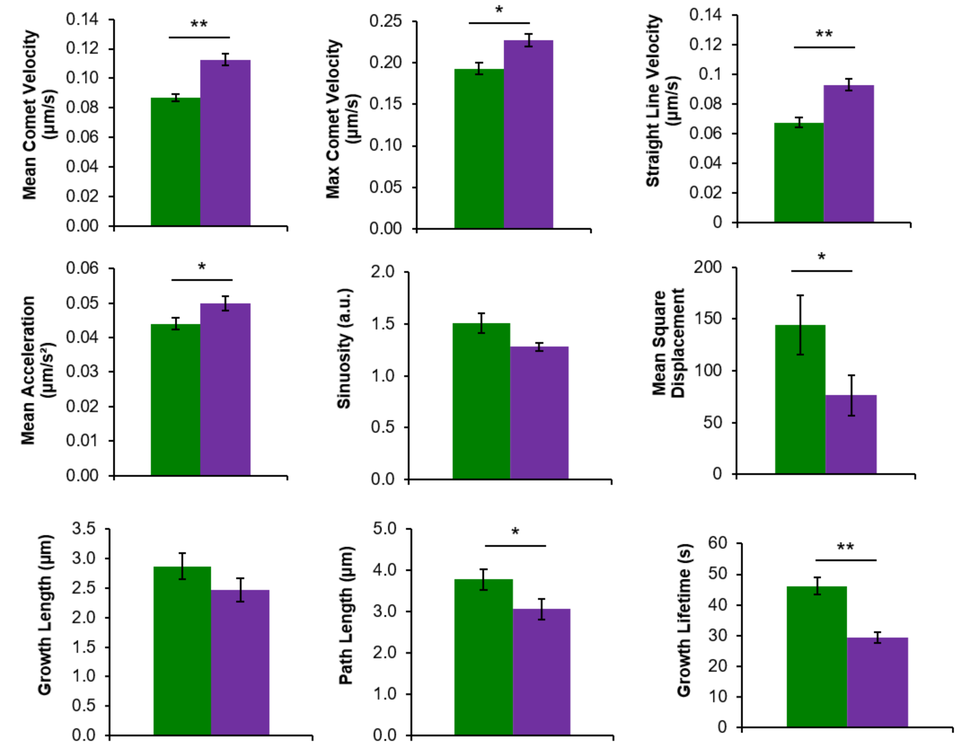

Figure 2. Comparison of tracking parameters in wild-type. EB1-GFP comets were analyzed in the sensory dendrites (green) or NMJs (purple) of wild-type *(elaV^{C155}-Gal4 x w1118)* larvae. Statistical significance was determined using the Mann Whitney U-Test (* $p < 0.05$, ** $p < 0.005$). EB1 comets at the NMJ showed higher "speed" associated parameters (mean comet velocity, max comet velocity, straight line velocity, mean acceleration), implying overall faster rates of MT growth in the NMJ than in the dendrite. However, EB1 comets in the NMJ showed lower values of mean square displacement, path length, and growth lifetime, implying that while NMJ MTs are "faster" compared to dendrite MTs, their growth is shorter and/or less stable.

Note: as a system validation experiment, we expressed EB1-GFP with two different sensory dendrite drivers, elav-Gal4 and 221-Gal4, and compared three parameters (mean comet velocity, growth length, and growth lifetime), observing significant differences between the drivers in all three parameters. This confirms the importance of a consistent Gal4-expression strategy.

Working Model

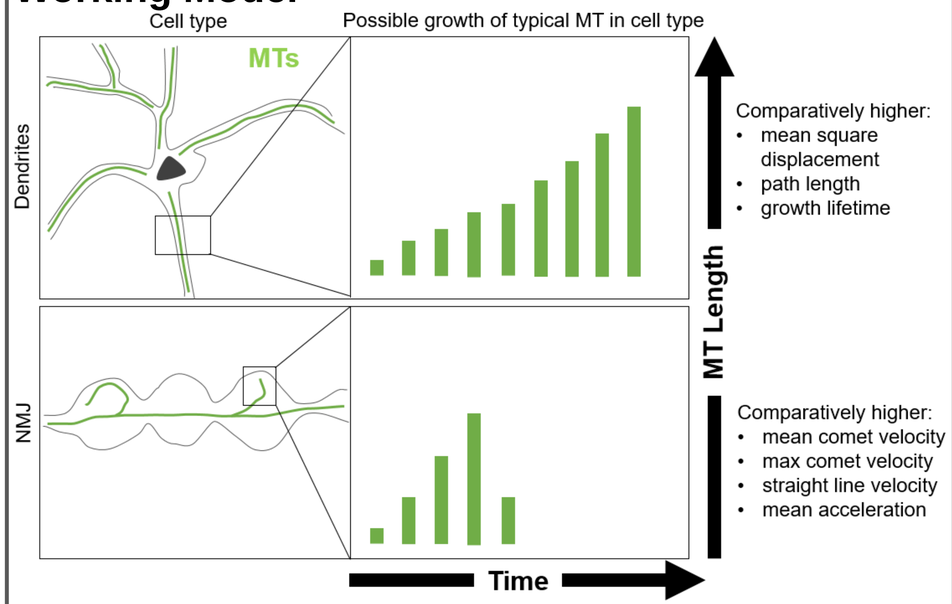

Figure 3. Comparison of MT dynamics in sensory dendrites and at the NMJ.

Conclusions

- We have demonstrated the utility of our new live imaging and analysis strategy for detecting differences in MT dynamics in different genetic backgrounds and cell types.
- We observed in sensory dendrites that EB1-GFP dynamics is dependent on the Gal4-driver, confirming the importance of a consistent expression strategy.
- NMJ MT growth dynamics were faster but shorter lived, compared to the slower but steadier growth of dendrite MTs.
- Certain parameters cannot yet be detected with our method, e.g. we cannot detect rescue events.

Future directions

- Apply our new method to determine the effect of known MT-associated proteins on the synaptic MT dynamics of the *Drosophila* NMJ.
- Develop two- and three-color approaches to correlate MT dynamics to other cellular structures, e.g. membrane, actin.
- Develop methods for quantifying additional parameters, e.g. shrinkage-related parameters and rescue events.