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Dynamic and Local Remodeling of Axon Caliber During Initial Myelin Ensheathment

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Dynamic and Local Remodeling of Axon Caliber During Initial Myelin Ensheathment

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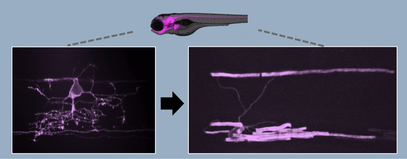
Introduction

During neural development, oligodendrocytes extend exploratory membrane processes that contact and wrap nerve axons with myelin. Our central goal is to learn how myelin sheaths are formed and stabilized during this time period.

Paradoxically, not all axons become myelinated. The mechanisms regulating this fate are poorly understood. In the larval zebrafish spinal cord, exploratory processes of single pre-myelinating oligodendrocytes span the entire dorsal-ventral axis. These cells have the potential to myelinate any spinal cord axon but only choose a select number of them.

We previously showed that neural activity biases axon selection for myelination. **In this study we ask:**

1. To what extent is axon selection specified by activity?
2. What are the activity-independent mechanisms of myelination?
3. Does the well-known correlation between axon diameter and myelination mean that diameter acts instructively and causally to specify axons for myelination?



1. Do zebrafish oligodendrocytes make stereotyped decisions to myelinate specific axons?

We used transgenic zebrafish with fluorescent reporters in specific subsets of neural cells to visualize axons and oligodendrocytes in different colors. We used confocal fluorescence microscopy to determine myelination fate *in vivo*.

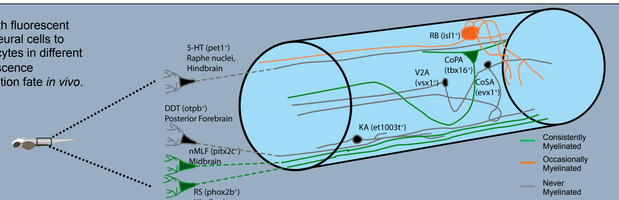
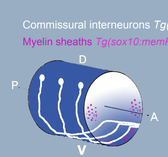


Figure 1. Summary of axon selection experiments. These findings are presented in full by Trudel et al. at the SFN FUN poster session (Monday 6:45-8:45pm, McCormick Place: North, Level 2 Mezzanine (Room: N228))

2. Is axon selection solely determined by neuronal activity?

The transgenic zebrafish line *Tg(evx1:GFP)* marks a subset of spinal cord commissural interneurons that are NOT selected for myelination. If axon selection is exclusively activity-dependent, we predicted that oligodendrocytes would ectopically myelinate *evx1*$^+$ axons when we suppressed electrical activity with tetrodotoxin.



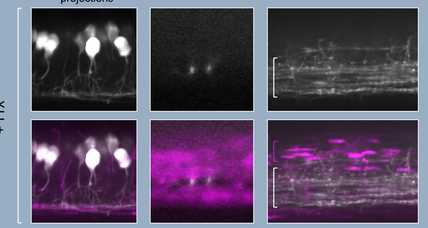
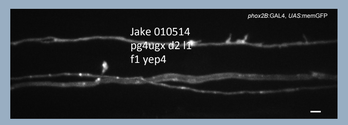


Figure 2. Suppression of electrical activity does not induce ectopic myelination of *evx1*$^+$ commissural secondary ascending interneurons.

3. Do oligodendrocytes preferentially myelinate high caliber axons?

If activity-independent, what are the mechanisms specifying axons for myelination? One predominant model involves axon caliber. Because axons that are myelinated are generally of higher caliber, it is assumed that these axons were selected for myelination due to their larger diameter.

Morphology of phox2B+ axons at later larval stages when myelination had already initiated. Were phox2B+ axons high caliber before myelination initiated? Scale bar 2µm.



We quantified the pre-myelinating diameters of numerous of myelin-fated and unmyelinated axons just prior to initial ensheathment.

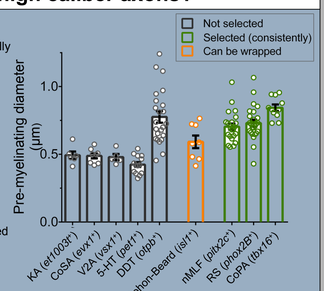
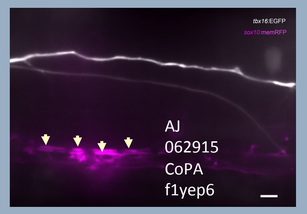


Figure 3. Summary of axon diameter measurements collected immediately prior to or at the initial onset of axon wrapping.

4. Does axon caliber predict the order that axons become selected for myelination?

If axons are selected based upon caliber, we further predicted that oligodendrocytes would select axons by ranking diameters. We systematically found the segment in which oligodendrocytes are initiating wrapping at 76hpf on 3 different subsets of axons.



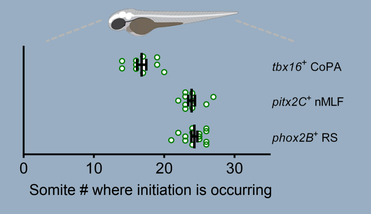


Figure 4. Temporal oligodendrocyte choice is not predicted by axon diameter. Representative fluorescence images show unmyelinated CoPA axons in the vicinity of oligodendrocytes wrapping other axon sub-types. Scale bar 5 µm. The summary graph shows that selection of nMLF and reticulospinal axons is developmentally advanced when compared to CoPA axons. We hypothesize that an intrinsic factor possessed by the axon determines myelination order.

5. Does axon caliber before initial wrapping predict myelination fate?

We tracked the diameter of individual *phox2B*$^+$ axons over the course of two days, beginning before initial wrapping. We predicted that higher caliber axons would be the first selected.

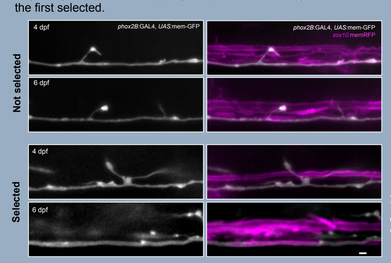


Figure 5a. Pre-myelination axon caliber does not predict myelination fate. Representative images show the same *phox2B*$^+$ axon at 4 dpf and 6 dpf. Axons in the upper and lower panels had similar diameters at 4 dpf, the axon in the lower panel became myelinated and grew radially at later stages. Scale bar 2 µm.

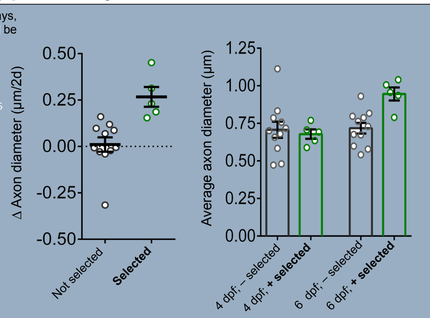
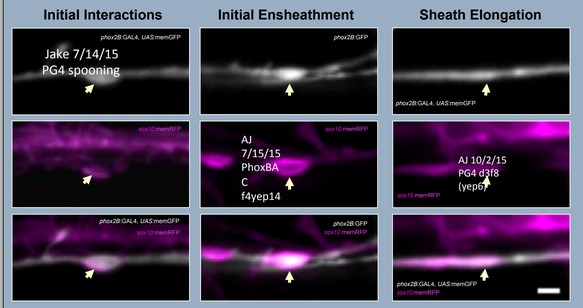


Figure 5b. Following the inception of myelination, chosen axons are higher caliber than those that haven't yet been selected. Summary measurements show the average diameters and extent of axon radial growth during the imaging experiment.

6. Oligodendrocytes interact with and wrap axonal varicosities.

During various imaging experiments, we have observed that the caliber of single axons is extremely variable. Oligodendrocyte interactions seem associated with the higher caliber domains known as varicosities.



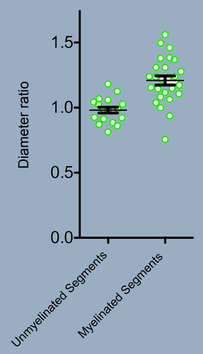
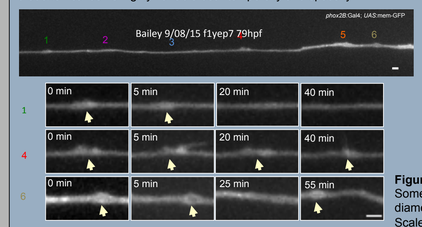


Figure 6. Axon-oligodendrocyte interactions at axonal varicosities. Representative images show the morphology of *phox2B*$^+$ axons at sites of initial interactions, initial wrapping, and ensheathment. Scale bar 2 µm. We quantified the diameter of axon segments at sites of ensheathment relative to neighboring, unwrapped segments to plot the local diameter ratio.

7. Are varicosities static or dynamic?

Given the drastic differences in axon caliber in a static image, we hypothesized that axon caliber is highly variable both temporally and spatially.



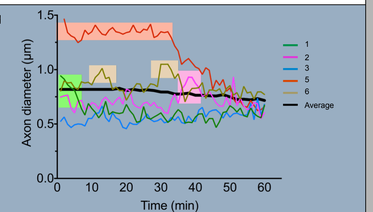


Figure 7. Axonal thickness is temporally and spatially differential. Some local domains can be static, but many are fluctuating in local diameter. Average diameter is essentially static over the same time period. Scale bar 2µm.

8. Is successful ensheathment dictated by changes in axon morphology?

Figure 8a. Are wrapping attempts at thin segments less stable? Timelapse shows a myelination attempt that is unsuccessful on a *phox2B*$^+$ that is fated for myelination. Note that local axon caliber is static during this interaction. Scale bar 2µm.

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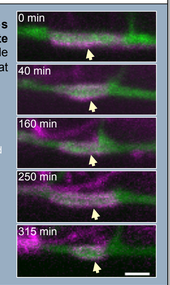
Figure 8b. Do axon varicosities promote stable axon-oligodendrocyte interactions? Timelapse shows a stable myelin sheath on a *phox2B*$^+$ axon that has formed around a varicosity. Scale bar 2µm.

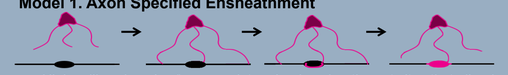
01/31/14 pg4 ugx 3d fast timelapse



Conclusions & Working Models

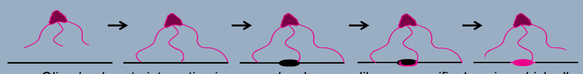
1. **Axon selection for myelination is not solely determined by neural activity**
2. **Average axon diameter prior to initiation of myelination correlates with myelination fate, but fails to predict it**
3. **When presented numerous myelin-fated axons, oligodendrocytes select axons in a specific temporal order**
4. **Among *phox2B*$^+$ neurons, the first axons ensheathed do not possess the highest caliber**
5. ***phox2B*$^+$ axons that are selected grow radially in congruence with ensheathment**
6. **Oligodendrocyte membrane processes congregate at local varicosities, which are temporally and spatially dynamic**

Model 1. Axon Specified Ensheathment



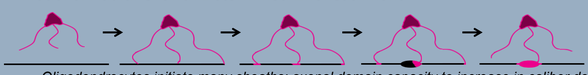
High caliber domains form pre-determined ensheathment sites that oligodendrocytes preferentially wrap.

Model 2. Oligodendrocyte Directed Ensheathment



Oligodendrocyte interaction increases local axon caliber on specific domains which allows stable sheath formation.

Model 3. Radial Growth Determined Ensheathment



Oligodendrocytes initiate many sheaths; axonal domain capacity to increase in caliber determines sheath stability.

Ongoing Experiments & Future Directions

1. Is axon diameter necessary or sufficient for axon selection?
2. What are upstream regulators of actin at local axon varicosities / ensheathment sites?
3. How do oligodendrocytes respond when axon availability is changed?
4. What are the molecules required for stable myelin sheath formation?

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