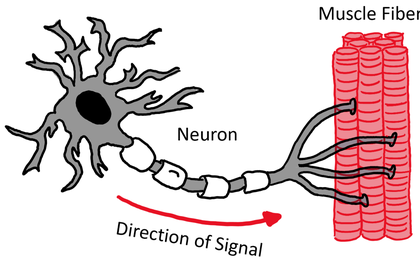
Utilizing Behavioral and Molecular Techniques to Study Gap Junction Channels in Zebrafish

Laura Reich[1], Rachel M. Lukowic[1,2], Adam C. Miller[1,2]

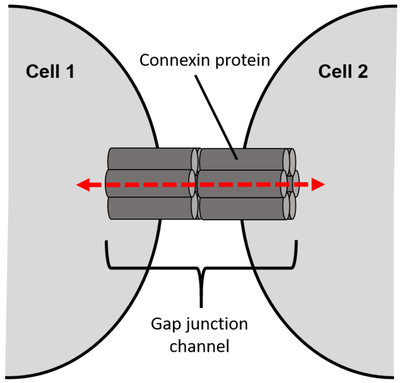
[1]University of Oregon, [2]Department of Biology

Background



The nervous and muscular systems coordinate activity to elicit motor behaviors

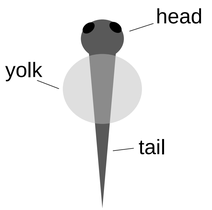
The nervous and muscular systems use gap junction channels (GJCs) to coordinate activity. GJCs are composed of connexins (Cxs) [1]



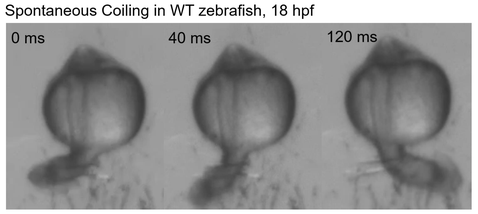
The Cx family is large and complex[2]

How do individual Cxs contribute to behaviors?

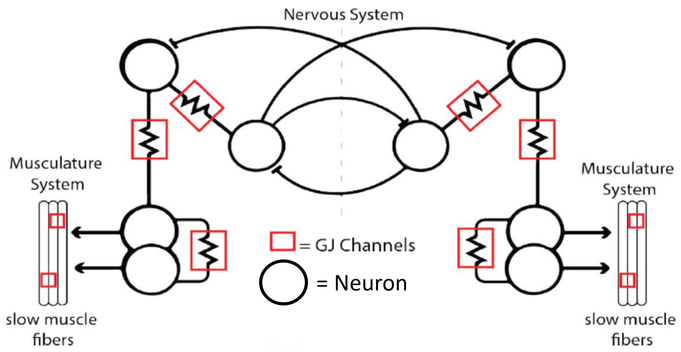
Embryonic Zebrafish



Spontaneous Coiling in WT zebrafish, 18 hpf



The Coiling Circuit



We can use embryonic zebrafish and its spontaneous coiling to identify Cxs in the coiling circuit that drive behavior[4]

Results

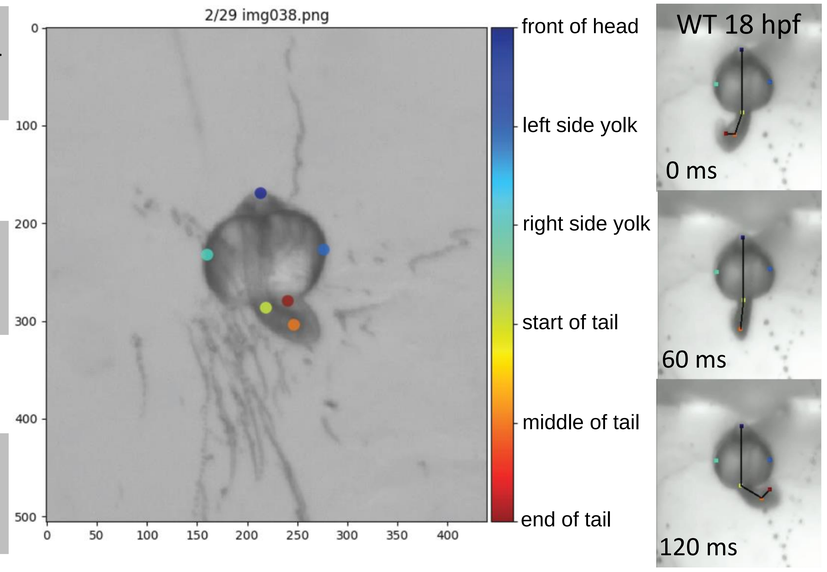
Develop an automated behavioral tracking system to screen for Cx mutants → Validate accuracy of system with WT and *cx46.8-/-*

DeepLabCut (DLC) [4] can be used to track coiling

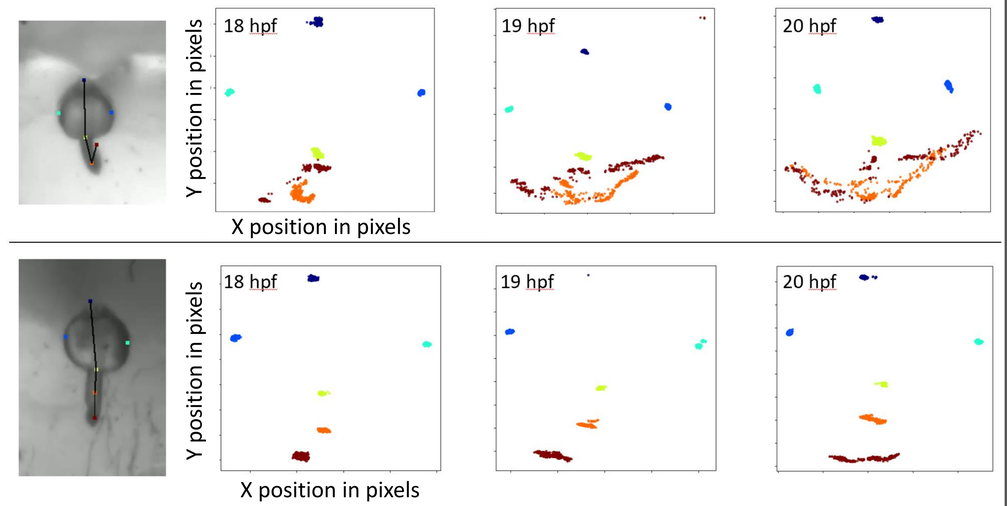
Capture 150 frames of WT coiling

Manually define points on frames

Train DLC network

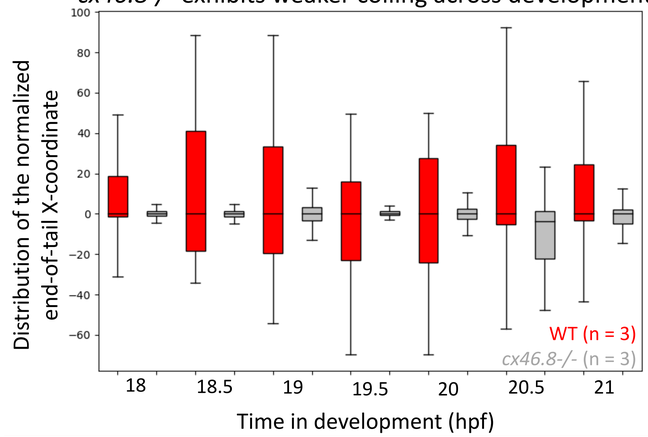


***cx46.8-/-* exhibit weaker coiling across development**



Results

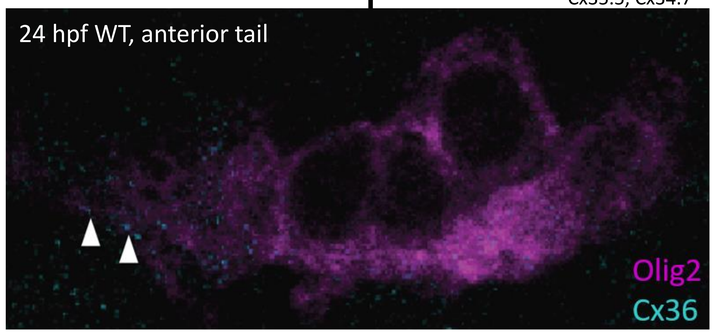
cx46.8-/- exhibits weaker coiling across development



Preliminary antibody staining suggests localization of Cx36 orthologs to coiling circuit neurons

Olig2 → Marks coiling circuit neurons

Human Cx36 marker → Recognizes Cx34.1, Cx35.1, Cx35.5, Cx34.7



Future Directions

1. Optimize DLC network
2. Antibody staining and RNA in-situ hybridization of neural Cxs and genes
3. Screen mutants of neural Cx-encoding genes

References

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Acknowledgements

Thesis Committee: Adam C. Miller, Rachel M. Lukowicz, Diana Libuda, Elizabeth Raisanen; UO Fish Facility: Tim Mason, Marcy McFadden; Help with DLC: Elliott Abe