Effects of Anesthetics on Mitochondrial Delocalization in Zebrafish Neurons

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Project Overview

Anesthetics such as propofol, etomidate, and ketamine are responsible for producing unconsciousness in patients. Mitochondria within neurons is responsible for energy dynamics, and its location within the neuron is critical. The effect of these anesthetics on mitochondria delocalization in neurons has not been properly investigated. Furthermore, transport proteins such as kinesin transport mitochondria to different parts of the cell. Previous studies have shown that kinesin motor proteins can be affected by anesthetics, altering the placement of mitochondria in the cell. By using a four fluorophore immunofluorescence stain, we can visualize different parts of the zebrafish brain. The use of a zebrafish model provides a model for exploring these critical research questions.

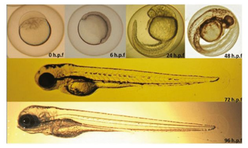

Figure 1: Zebrafish embryo development
Reference 1

Methods

1. **Dissection of Zebrafish Brains:** Using a dissecting microscope and fine forceps, we dissected out zebrafish brains to use for both cell culture and immunofluorescence staining.
2. **Whole Fish Staining:** We moved from staining zebrafish brains to staining whole fish for better visualization. We used four different stains, each targeted towards a different part of the neurons.
   a. **Antibody Stains**
      i. SNAP-25: targets the synapses of the neurons. Appears red.
      ii. Acetylated tubulin: targets the tubulin network of neurons. Appears green
   b. **Chemical Stains**
      i. DAPI: Targets the nuclei of neurons. Appears blue.
      ii. Mitobrilliant: Targets the mitochondria of neurons. Appears purple.
3. **Immunofluorescence Microscopy:** Using the Keyence microscope (immunofluorescence microscope), stitching, and z stacks, we were able to compile overlaid images.
4. **Kymograph Analyzation:** Using ImageJ, we tracked the movement of mitochondria down the axons. From this, we were able to analyze retrograde (movement towards the cell soma) and anterograde (movement towards the synapse) transport of mitochondria. This information will be helpful in determining anesthetic effects of mitochondrial delocalization in neurons.

Results/Images

Figures 2-6: Quadruple immunofluorescence stain highlighting different parts of the zebrafish neuron within a whole fish

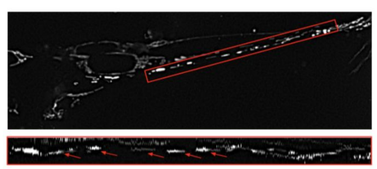

Figure 7: Mitochondria movement down an axon that will get translated into a kymograph

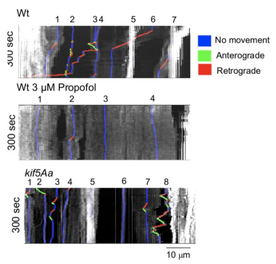

Figure 8: Tracks showing the movement of mitochondria down the axon

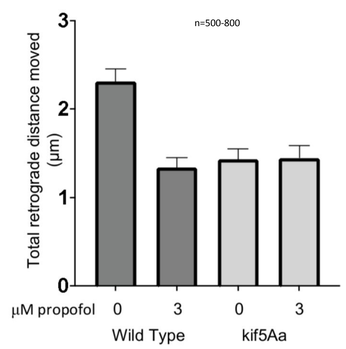

Figure 9: Data from retrograde movement of mitochondria

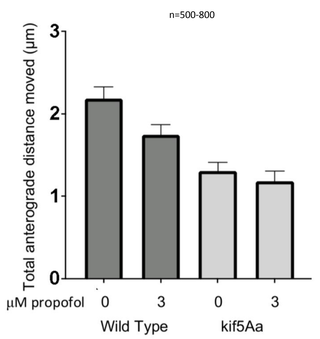

Figure 10: Data from anterograde movement of mitochondria

Next Steps

1. **Use of agarose gel for mounting**
   a. In order to prevent movement during imaging, agarose gel channels might be helpful. By positioning the embryos belly side up in the gel, movement during imaging will be limited, improving the quality of data collected.
2. **Sectioning of tissue**
   a. To better visualize neural networks in zebrafish, it might be helpful to section tissue instead of using whole fish. Sectioning allows better single cell visualization. To accomplish this, we might use resin or paraffin with cryosectioning.
3. **Analyzation of kymographs**
   a. Continue to analyze mitochondria movement within axons to determine delocalization of energy storage under anesthetic influence.

References

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2. Santos D, Monteiro SM, Luzio A. General Whole-Mount Immunohistochemistry of Zebrafish (Danio rerio) Embryos and Larvae Protocol. Methods Mol Biol. 2018;1797:365-371. doi: 10.1007/978-1-4939-7883-0_19. PMID: 29896703.

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