The Effects of Bisphenol A (BPA) on the Neural Development of the *Xenopus laevis* Tadpole

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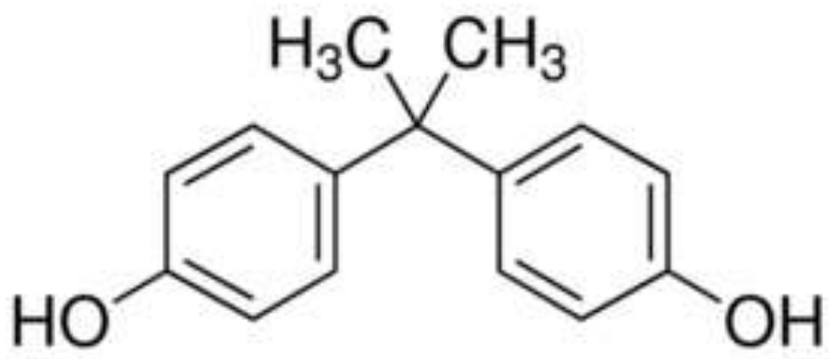
Overview

- Introduction & Background
- Visually Guided Behavior Study
- Electrophysiology Studies
- Axon Circuit Development Study
- Conclusion
- References

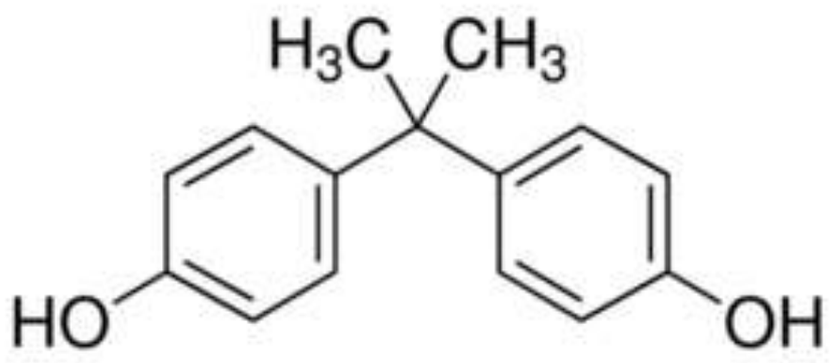
Introduction

- Bisphenol-A
  - Common chemical compound used in manufacture of plastics and canned goods
  - Known endocrine disruptor
  - 2 phenol rings - estrogen mimic



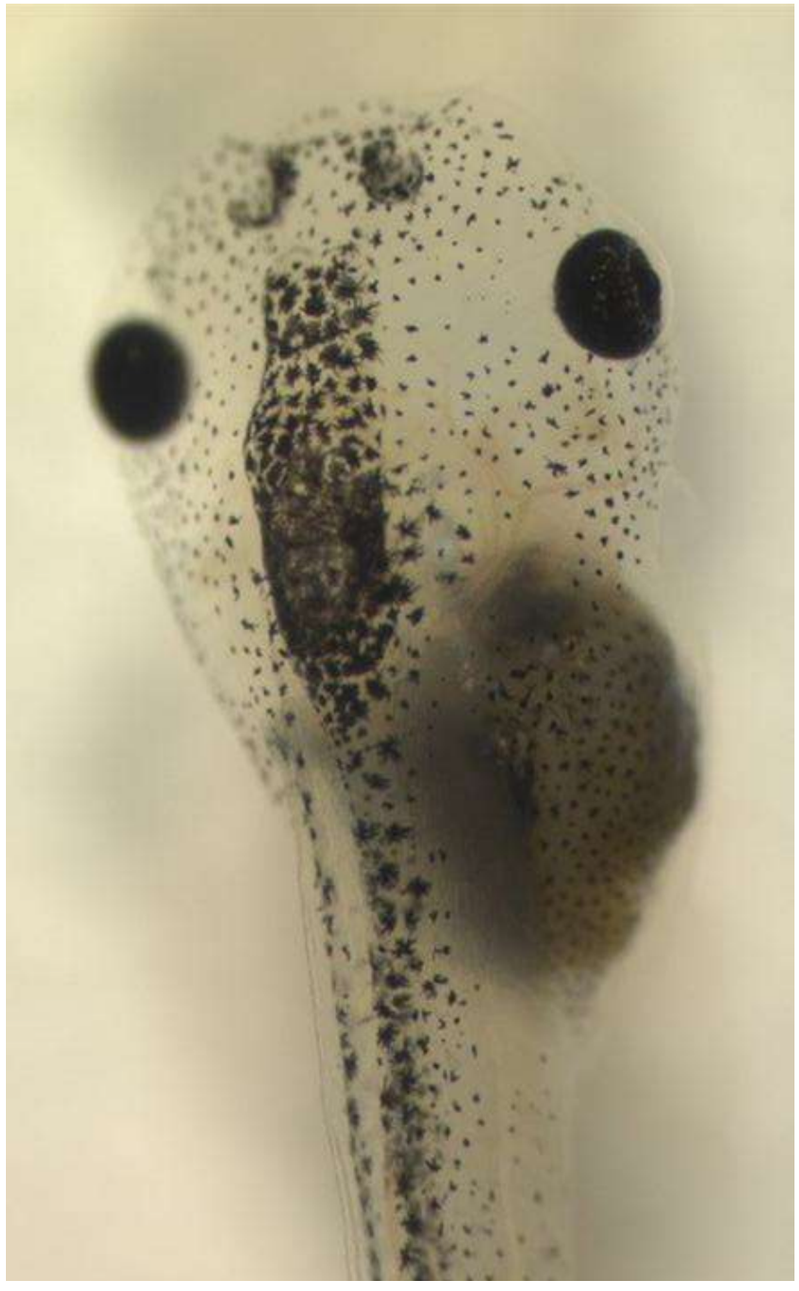
Introduction

- Bisphenol-A
  - Many studies show BPA has effects on adult mammalian nervous system
  - We are investigating BPA’s effects on a developing neural circuit

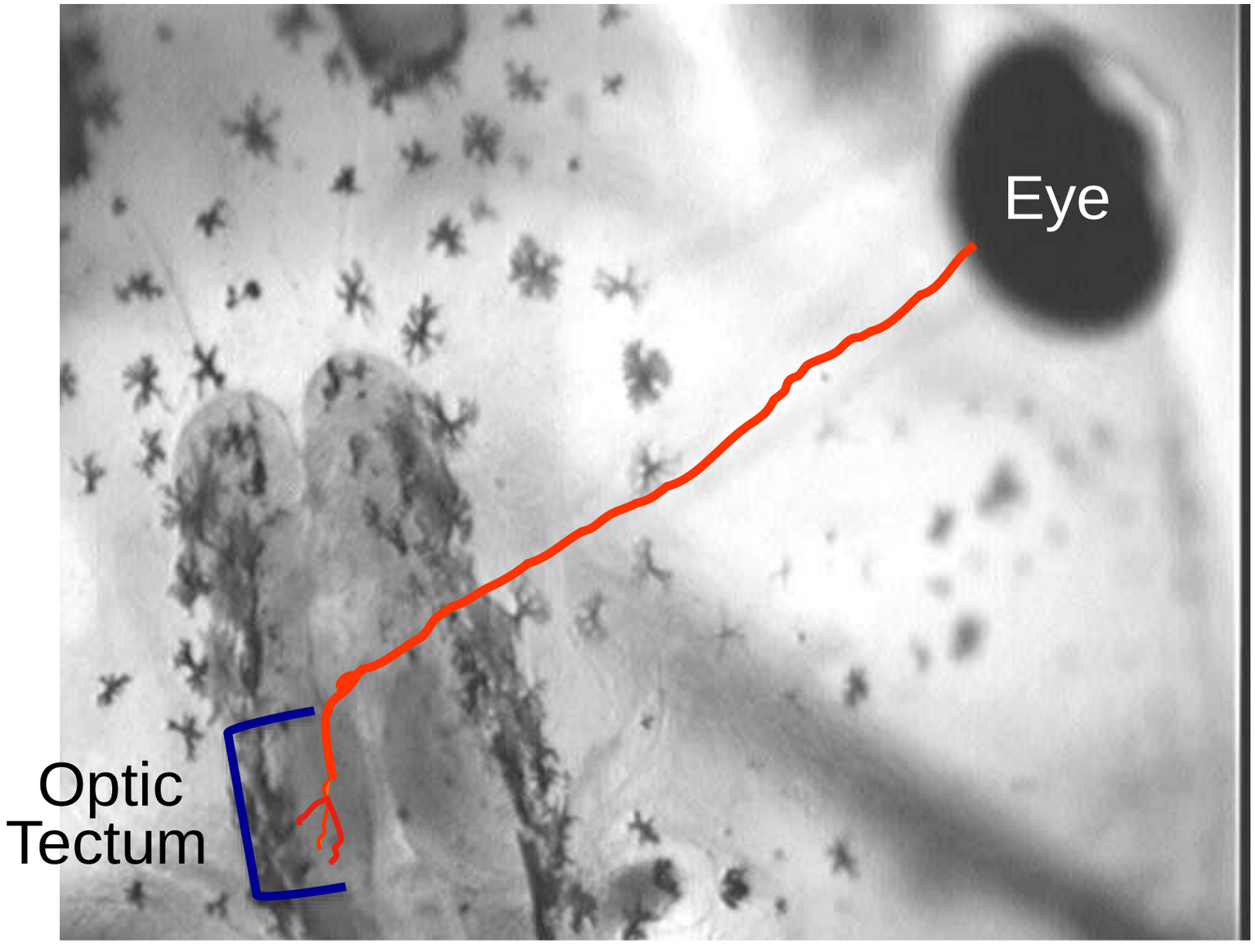


Introduction

- Why *Xenopus*?
  - Mature in relatively short time period
  - Many tadpoles per fertilization
  - Well-documented stages of development
  - Simple visual circuit model
  - Relative ease in toxin exposure and dose control



The Retinotectal is the major circuit of the tadpole visual system



Introduction

Pratt Lab - Goals

- Expose *Xenopus* to 10 & 15 µM BPA in Steinberg solution
- Divided into three parts:
  - ★ Behavior study
  - ★ Electrophysiology study
  - ★ Imaging study

Research Design

•Groups of 10 tadpole embryos were selected 12 to 24 hours post-fertilization.

•Each group was assigned to either a control, 10μM, or 15μM bowl and placed in their respective concentrations.

•The concentrations were optimized at 10 & 15 μM.

•100mL of Steinberg Solution in glass bowls was the synthesized habitat for the tadpoles.

Research Design

•For the first half of the lab's experiments, the tadpoles were observed every third day.

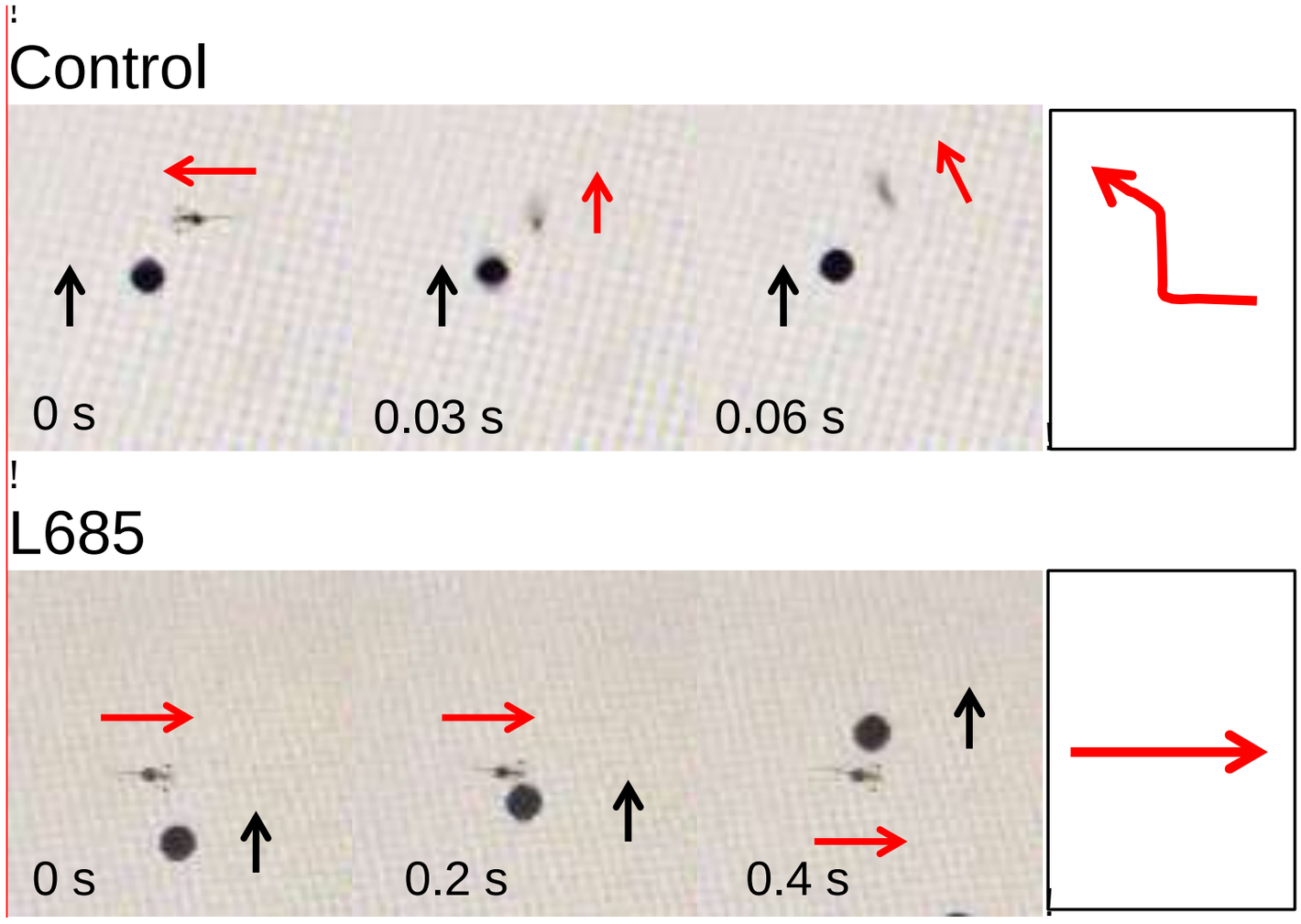
•The control and BPA solutions were refreshed every third day, using the same BPA stock for the entirety of those groups' respective experiments.

Behavioral Test Design

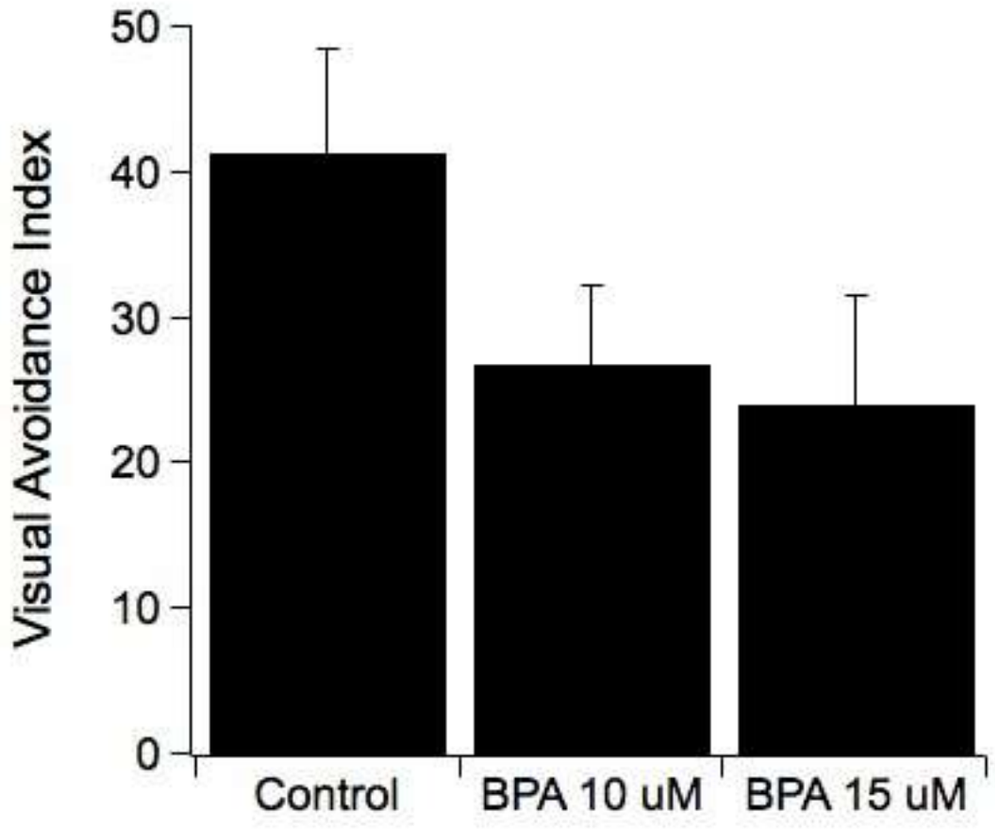
•Behavioral tests were conducted 8-10 days post fertilization.

•The tadpoles were recorded for three minutes, the video was analyzed for encounters the tads had with the dots.

•Five encounters for a tadpole are necessary in order for the information to be used.



Results


Visual Avoidance Index
50
40
30
20
10
0
Control
BPA 10 uM
BPA 15 uM

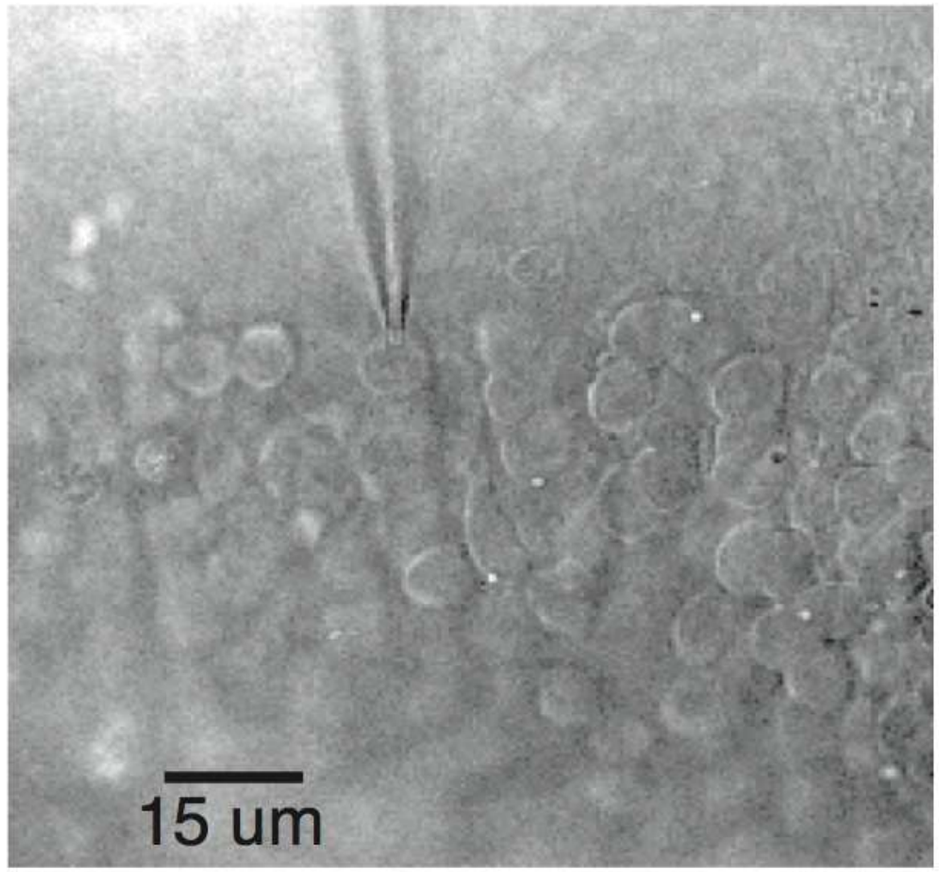
Electrophysiology Test Design

- Tadpoles at stage 48/49 were selected,10 days post fertilization, from the 15uM concentration.

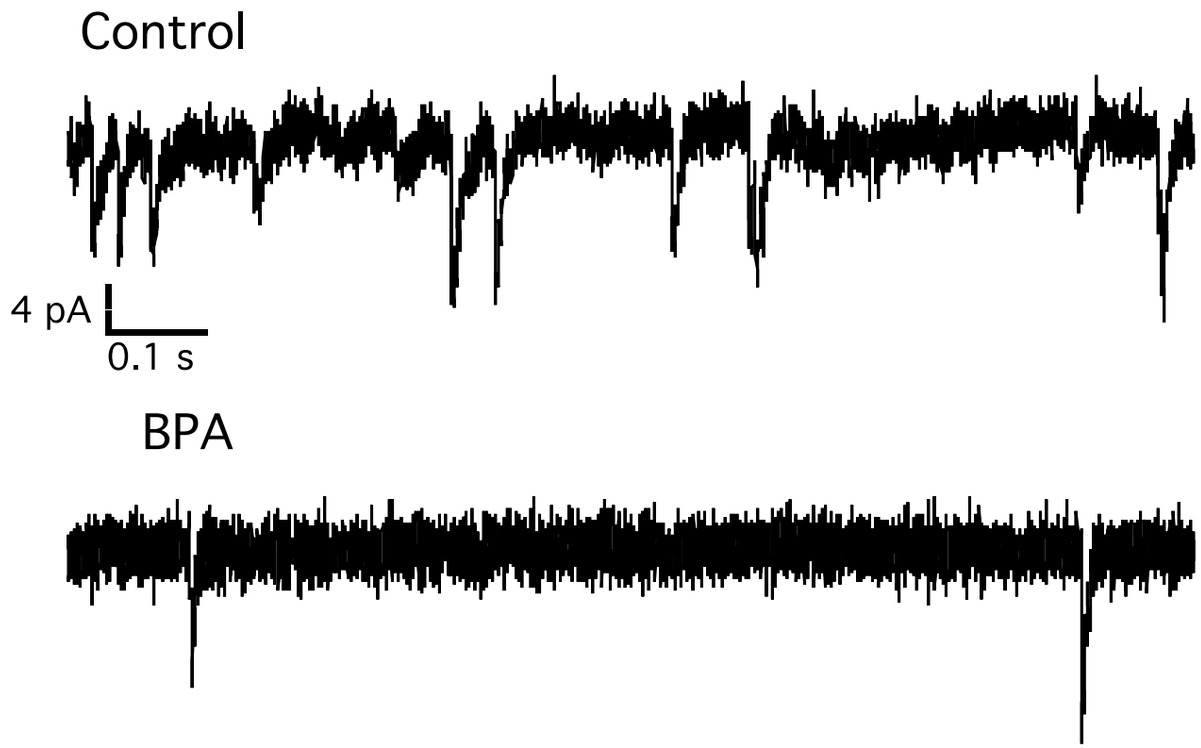
- Whole brain preparation

- Record from tectal neurons using a glass micropipette

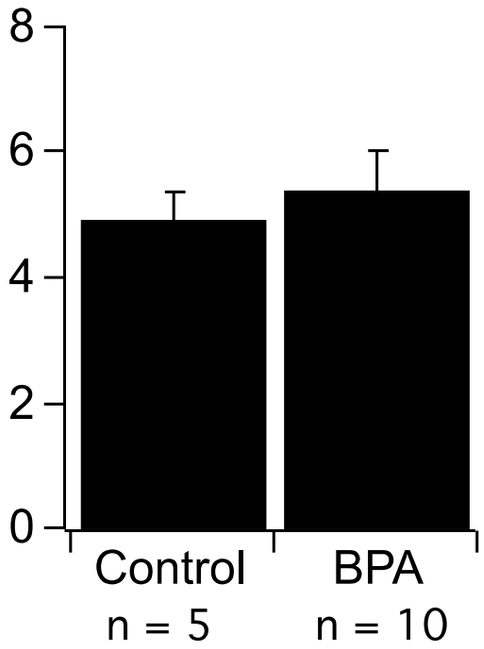
- Recorded spontaneous Excitatory Postsynaptic Currents
  - Spontaneous activity in the brain.
    - Amplitude=Strength of Synapses
    - Frequency=Number of Synapses

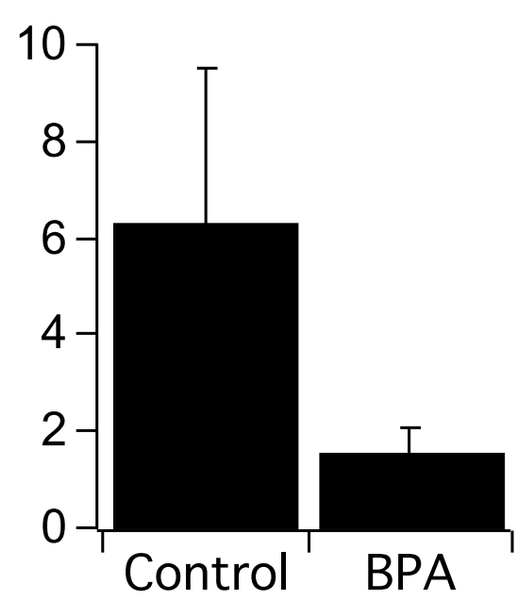


Electrophysiology Results



These are 1 second traces of recordings of the spontaneous Excitatory Postsynaptic Currents (sEPSCs).





Using Fluorescent Dye to Image Visual Circuit Axons

- Injections are done using stage 44 tadpoles
- Tadpole set up on dissecting scope stage after MS-222 Immersion
- Glass pipette filled with Dye-I and injected into eyeball of tadpole
- Melanocytes on top of brain are removed by scraping to allow imaging of axons entering tectum
- Tadpole placed in formaldehyde fix for one week and then imaged using confocal microscopy

Zeiss 710 Confocal Microscope

- Provided by University of Wyoming Jenkins Microscopy Facility

Results

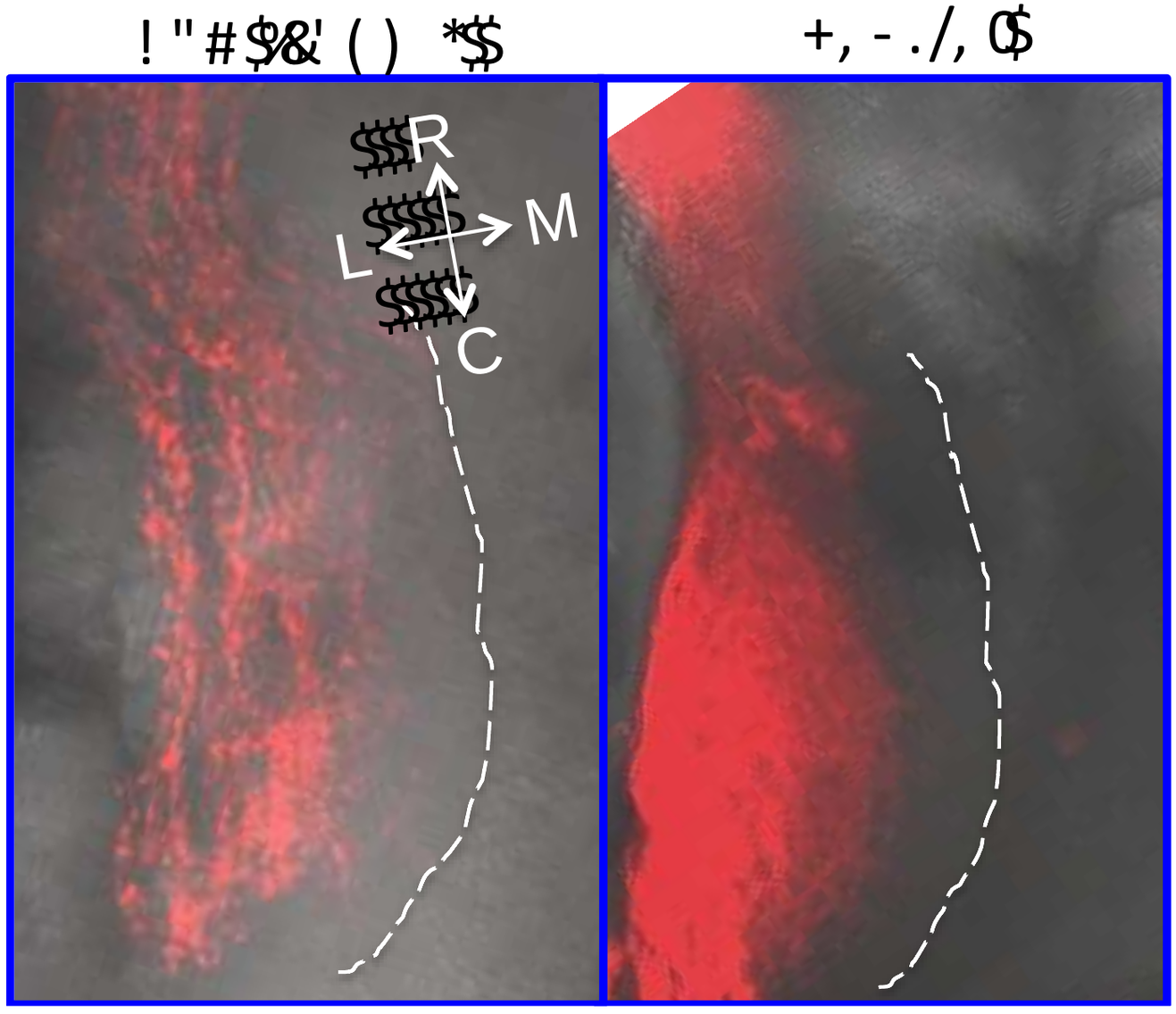


Fig. 1 - Control and BPA exposed *Xenopus laevis* tadpole retinal ganglia axons traveling to optic tectum of brain.

Future Research

- There are significant differences between BPA tadpoles and control tadpoles at the behavior, cell, and circuit levels.
- The Pratt Lab has optimized testing the visual system of tadpoles, therefore, tests at the circuit, cell, and behavioral levels will continue.
    - Every two weeks, new groups of tadpoles are tested.

Thank You

- We would like to thank INBRE and EPSCoR
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