The Rapid Effects of Testosterone on Visual Stimuli in Male Goldfish
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My research this summer focused on how sex-steroids affect sensory systems in animals. Since they work in a conserved system of regulation, what I learned about sex-steroids in goldfish may be applied to other animals. Sex-steroids have two ways of affecting social behavior in animals. In the genomic mechanism, which has been most studied, steroids diffuse through the cell membrane and serve as a transcription factor for certain genes in the nucleus of the cell, thus affecting behavior. However, there are also non-genomic mechanisms by which sex-steroids affect behavior in animals. Through these mechanisms, they attach to receptors on the cell membrane that send off secondary messengers and alter animal behavior. While the genomic mechanism may take months to affect the animal, non-genomic mechanism may take only take hours. My project focused on whether the sex-steroid testosterone can rapidly affect social behavior in male goldfish in response to visual stimuli.

In order to determine if testosterone rapidly affects social behavior in response to a visual stimulus (female goldfish), I developed an ideal experiment in which each male goldfish would serve as its own control. This would reduce variability among the male goldfish that were tested. Each male goldfish would wear an eye patch over one eye, so it couldn’t see the female goldfish out of the covered eye, but would be able to see the female goldfish out of the other eye. They would sit in a tank overnight and in the morning, be exposed to (but only be able to see) a female goldfish on each side of its tank for an hour and a half. The male goldfish’s brain would then be removed, and the number of cells containing c-fos proteins in each half of the brain would be counted. C-fos proteins are proteins that may be indicators of visual activation in the brain. This experiment would work in theory because 99% of the visual input from one eye goes to the opposite side of the brain. A significant difference in the number of cells with c-fos proteins in different sides of the brain would indicate that testosterone can rapidly affect visual activation in response to a stimulus.

However, before the ideal experiment could be performed, I had to create an eye patch for the male goldfish and determine whether c-fos protein is a marker of visual activation in the optic tectum (the part of the brain where visual information is processed). When working on an eye patch that would need to stay on a moving goldfish overnight, I tried to use Gluture veterinary glue, crazy glue, boat glue, and reef glue to attach the eye patch. I used these glues because I read books on fish surgeries that suggested them for use on fish. However, while the glue would often stick to immobilized fish, they would not stay on the fish while it was moving. I tried to use the pipette tips, aluminum foil, and cloth to construct the patch. Only one patch model was successful in staying on moving fish overnight, which was the cloth patch attached with reef glue. However, it only worked on two fish. One major obstacle in constructing the eye patch was that the goldfish’s eyes were too close to their mouths, gills and nostrils and would endanger their health if too much glue were applied or it got into any of these areas.

I also needed to determine whether c-fos protein is a marker of visual activation in goldfish. I placed male goldfish in individual tanks overnight and in the
morning, removed half of the ones I tested from their tanks without turning the lights on and removed their brains. I then turned on the lights and surrounded the other half of the male goldfish that were tested with female goldfish that they could only see on each side of their tank for an hour and a half, after which, their brains were removed. The brains were stored in 4% paraformaldehyde solution for an hour and a half and then moved to a 30% sucrose solution overnight. The next day, they were frozen and cut into 20 micrometer slices using a cryostat and placed on slides. An immunohistochemistry was run on each brain. Immunohistochemistry allowed me to visualize c-fos proteins by attaching primary and secondary antibodies to c-fos proteins, causing them to glow. I then calculated the density of cells containing c-fos proteins by drawing a circle around the area containing those cells and dividing by the number of counted cells containing c-fos proteins. Initially, I looked at the superficial layers of the optic tectum part of the brain and there appeared to be no difference between the density of cells containing c-fos protein in fish that were exposed to light and females and fish that had been exposed to darkness. However, when looking at the SPV layers of the optic tectum (where sensory input is integrated into motor output), there appeared to be a difference between fish that had been exposed to light and females and those that had been exposed to darkness. Although there weren’t enough test subjects to run statistical analysis on the densities, my findings suggest that c-fos may be a marker of visual activation in the optic tectum.