The function of sex steroid in vertebrate animals is to modulate a variety of behaviors, especially those implicated in mating and courtship activity. Sex steroids like testosterone and estradiol have long been known to be able to diffuse into cells passively as a result of their hydrophobicity. It is well known that these hormones have genomic effects in the cells into which they diffuse. That is to say, when the hormone binds to its receptor inside of the cell, the hormone-receptor complex activates genes in the nucleus and ultimately affects protein production. This mechanism of action typically takes hours to days to elicit any sort of behavioral response, as a certain amount of protein much accumulate before any changes occur.

The role steroid hormones play in more rapid cellular and behavioral modulation has been of great interest. Steroid hormones are capable of binding to membrane bound receptors, activating secondary messenger pathways inside of the cell. Those secondary messenger pathways cause the cell to rapidly change its physiology (Lösel and Wehling, 2003). However, we know little about how those rapid changes may rapidly alter the behavior of animals.

A recent study has shown that testosterone or estradiol injections rapidly increased visually guided sexual behaviors of male goldfish towards female fish (Lord et al., 2009). Specifically, the injections increased the time a male goldfish spent in proximity to a female stimulus separated by a Plexiglas barrier that prevented all but visual communication. These effects were evident in fifteen to thirty minutes, a time course faster than those typically associated with the genomic actions of steroid hormones.

That injections of sex hormones rapidly modulated visually guided behavior suggests that the mechanism of action of these hormones may be in the visual system of the male goldfish. One area of interest is the retina, the part of the visual system that first receives sensory information from the environment. Previous research has shown that an estradiol receptor, ERβ, is present in certain layers of the goldfish retina (Michaud, 2013). Importantly, it seems that the ERβ found was not localized in the nuclei of the cells where hormone receptors are classically found, but rather along the long processes, suggesting that the receptors may have some function outside of their normal genomic function.

The research I conducted this summer was designed to further our understanding of how estradiol might modulate visual behavior at the level of the goldfish retina. I used electroretinography to investigate this. When exposed to light, the cells of the retina produce a characteristic and stereotyped burst of electrical activity. Using certain kinds of electrodes, the sum of this electrical activity can be recorded and presented in a waveform called an electroretinogram (ERG). An ERG has several different components that result from certain cell types within the retina (Figure 1). One of these components, the b-wave, is an indication of ON-bipolar cell activity. The amplitude of this part of the ERG can be used as a measure of the sensitivity of the retina to a given light stimulus. Prior research has shown that in an \textit{in vivo} preparation, injections of estradiol significantly increased the b-wave amplitude.
produced in response to light in male fish (Schwemberger, 2012). However, it was not determined if ERβ mediates this response.

To determine the receptor that mediates rapid estradiol effects on retinal physiology, I attempted to further develop an in vitro eyecup preparation onto which I would be able to directly infuse ERβ antagonists to see if they could block estradiol's physiological effects. To prepare this set-up, a dark adapted male goldfish is first euthanized, then the eye is removed. Next, the cornea and lens are removed, along with as much of the vitreous fluid as possible, leaving the bottom hemisphere of the eye containing the intact retina. The remaining eyecup is placed in saline in a dark room and exposed to flashes of a white LED light of varying intensity. The resulting retinal activity was recorded with extracellular electrodes. The majority of the summer was spent investigating different ways of minimizing electrical noise and maximizing sensitivity to any retinal response. This involved testing different kinds of extracellular electrodes, reducing vibrations and airflow in the testing room, changing amplification settings and changing the placement of the electrode within the eyecup preparation. Also, I worked to find a method of better preserving the eyecup by assessing the preparation's viability in different varieties of saline, and bubbling oxygen through the saline it was sitting in. By the end of the summer, however, it became obvious that instead of measuring retinal responses to light, the electrodes were measuring a photovoltaic effect of the light stimulus. This was discovered when I testing the integrity of one of my extracellular electrodes by recording from a dish containing only saline. This allows me to see the baseline high frequency noise that particular electrode conducts. When I flashed the LED light on the electrode in the dish containing only saline and no eyecup preparation, I found that it produced a waveform that very closely resembled the stereotyped ERG wave seen in Figure 1, but had a few subtle differences in shape. It then became apparent that all of the waveforms recorded over the course of the summer assumed the slightly different shape of the photovoltaic response, instead of the typical ERG response.

Future research will reinvestigate the possibility of using an in vivo preparation to try and establish a dose-response relationship between estradiol and b-wave amplitude changes. Certain kinds of electrodes can be used that allow for both a more consistent recording from a live fish, and do not respond to the light stimulus being presented to the fish. Additionally, there is more immunohistochemical work to be done that could determine whether or not the estrogen receptors found in the retina are membrane bound or located cytoplasmically. Work can also be done to better determine which retinal cells actually house the estrogen receptors found. The role of dopamine in retinal visual sensitivity is also a question to be investigated.
Figure 1. Stereotypical electroretinogram recorded from a goldfish retina. The b-wave is representative of ON-bipolar cell activity, and the amplitude can be used a measure of visual sensitivity of the retina.

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References