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Research Proposal:

Using Electroretinography to Investigate Rapid Sex Steroid Effects on Neuronal Activity in *Carassius auratus* Retina

Sex steroid hormones, including androgens and estrogens, have a wide array of cellular effects. Traditionally, steroid hormones have been considered slow acting, a consequence of mechanisms through which the expression of various genes are altered due to steroids binding to receptors that interact with the DNA. Genomic mechanisms thus typically affect future behaviors by setting up the neural systems involved in behavioral regulation. Recently, a more nuanced understanding of steroid hormones' mechanisms of action has been widely adopted with the discovery of rapid, non-genomic mechanisms. Rapid, non-genomic mechanisms mean that fluctuations in concentrations of steroid hormones could immediately change an organism's behavior on a minute-by-minute basis. With the identification of rapid pathways in addition to the traditional genomic pathways, steroid hormones are now recognized as more dynamic modulators than ever before, yet not much is known about what neural systems are affected and what the subsequent changes in behavior may be. Preliminary research has established some behavioral changes in a couple of species, but much more research is needed to establish what areas and pathways of the brain are active in mediating these behavioral changes in different organisms. I will work in the Thompson lab in collaboration with Professor Thompson and Professor Dickinson to determine where in the brain sex steroid hormones, specifically estrogens, exert rapid, non-genomic effects on goldfish behavior.

In recent years, the *Carassius auratus* Goldfish has been studied in the Thompson lab in order to learn more about neuroendocrine mechanisms that control social behavior. Previous studies have indicated that after male goldfish are exposed to female fish, there is an increase in testosterone levels in male goldfish. Follow-up experiments conducted in this lab have observed that injections in male

goldfish of testosterone or estradiol, a type of estrogen, rapidly change sexual approach behavior towards females, presumably through a non-genomic mechanism. However, it is not yet known where in the brain these effects are mediated (with changes perhaps occurring in sensory systems, motivational systems, or motor systems). It is also not known via what mechanisms the rapid effects might be mediated, but some have been proposed. I plan to help answer the question of where in the brain, and thus what brain systems are involved in mediating these rapid effects. We plan on starting the process by focusing at the retina, photosensitive neural tissue at the back of the eye involved in the sensing and processing of visual stimuli from the environment. We want to identify whether or not estrogens rapidly alter the way the retina processes visual inputs in order to see if this neural tissue is involved in the observed behavioral changes. An enzyme known as aromatase converts testosterone into estradiol, via a process called aromatization. Previous studies in Gloria Callard's lab demonstrated that aromatase levels are high in the neuronal fibers of the visual system, including the retina. The large amounts of aromatase in the retina suggest the retina's involvement in such rapid estrogen related behavioral changes.

To answer this question of the retina's involvement in the mediation of these rapid pathways, we need to detect any changes in retinal sensitivity to light. To do this we will use electroretinography where an electrode placed near the retinal cells will record the voltage changes that occur when they are exposed to a light stimulus. The retinal response to light has a stereotypical voltage change associated with it. Specifically, the amplitude of the b-wave, which corresponds to on-bipolar cell responses, tends to increase in proportion to the intensity of light. I will have a group of fish treated with estradiol and a group given a control. In a time period before genomic mechanisms would be able to exert an effect we will analyze whether or not the treatments of estradiol change the level of light sensitivity of a male retina. We intend to use a novel *in vitro* eye-cup preparation that will allow us to more easily manipulate the local drug environment than has been previously possible. We will thus be able to use

this setup to conduct experiments to determine which kind of estradiol receptor may be mediating the change in visual sensitivity in the presence of estradiol. Recent findings have shown that the ERbeta estradiol receptor is distributed throughout goldfish retina. We will block the activation of this receptor by applying an ERbeta antagonist to see if the effects of estradiol are diminished. If we do find significant alterations in the photosensitivity of the retina in response to treatment with estradiol, this would support a conclusion that sex steroids in goldfish can rapidly alter behavior and do so, at least in part, by changing the processing of visual stimuli. If we do not detect differential photosensitivity, future work would follow up by analyzing activity patterns in other pathways in the goldfish brain. The results of this research will help to direct the focus of future research investigating the molecular basis of these effects and provide a starting point when looking at pathways sex steroid hormones affect in other species.