Sex steroid hormones are capable of migrating a great distance from their source to act on target tissue and organs, including neurons in the brain, where they subsequently influence a variety of functions, such as behavior. Classically, steroids bind to specific intracellular receptors and act as ligand dependent transcription factors to turn genes on and off (Falkenstein et al. 2000). However, it can take hours to days for steroids, to set up systems, including brain circuits that support behavior. In the Thompson Lab we have seen that estradiol (E2), can elicit fast-acting behavioral responses in male Carassius auratus, freshwater goldfish, too rapid to occur through classical, genomic mechanisms (Lord et al. 2009). Evidence suggests that the rapid mechanisms of E2 are mediated by membrane bound estrogen receptors (ERs), however, the identity of such receptors is unknown. The aim of my work this summer was to provide a more complete picture of the receptor mechanisms mediating rapid, non-genomic actions of E2 in the brain of male goldfish.

We conducted E2-receptor bindings assays both in vitro, dead tissue and performed E2 infusions, in vivo, live goldfish. E2 was conjugated to a fluorescent marker and bovine serum albumin (BSA), a hefty, membrane impermeable protein that is often used to characterize membrane-binding. We used milting and non-milting male goldfish in the in vitro and in vivo paradigms.

In the in vitro binding assay fish were sacrificed and brains were extracted. Brains were fresh frozen, then sectioned into 60um, 100um and 120um sections. Sections were placed onto subbed slides, which allowed the tissue to adhere to the glass better than untreated slides. After placing the tissue onto the slides, half of the slides were fixed with a light fixative. First, the tissue was treated with E2/BSA/FITC (1mg/ml in ethanol) followed by an Alexa 488 / anti-FITC marker.

The purpose of the in vivo infusions was to provide an environment for the steroid conjugate to circulate throughout the brain, and potentially, bind to existing membrane-bound receptors. The target site of infusion was the third ventricle, located between the telencephalon and optic tectum, both of which are critical brain regions of the visual system. The anaesthetized fish was placed in a stereotactic device. Next, a small, circular expanse of skull surrounding the target site was excised with a surgical drill and forceps. When the target site was discernable, a 5ul Hamilton syringe was employed to deliver the E2/BSA/FITC (1mg/ml in ethanol). The Hamilton syringe was placed into a micromanipulator device and the head of the syringe was lowered approximately 1mm below the surface of the target site before the E2/BSA/FITC was discharged. Two hours following the steroid conjugate infusion the brain was extracted, then fixed in paraformaldehyde.

We utilized fluorescent microscopy to analyze the tissue obtained from the in vitro binding assay, as well as the in vivo infusions. The results from the E2/BSA/FITC binding assay clearly showed nuclear, and potentially, non-nuclear signal in the telencephalon, pre-optic area and optic tectum. Specifically, in the Supraopticparaventricular (SPV) region of the tectum we noticed an interesting pattern of signal that did not appear to be nuclear. This staining may represent membrane-bound ERs binding the E2/BSA/FITC. Additionally, in the in vivo infusions we observed non-nuclear signal, distinct from that observed in the in vitro binding assay. This bright punctate staining was most pronounced in the olfactory pathway and areas of the optic tectum. Interestingly, recent evidence suggests that the olfactory system plays a role in social approach behaviors in male goldfish.
The results from the in vitro binding assay and in vivo infusions suggest that non-nuclear, membrane bound ERs may be present in the goldfish brain. Furthermore, the presence of these membrane bound receptors may directly influence the rapid effects of E2 on behavior. In light of the current data, further investigation is required in order to validate that the observed signal highlights membrane-binding patterns of ERs in the brain.

Works Cited
