Investigating the Rapid Effects of Testosterone on Olfactory Processes in *Carassius Auratus*
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Project Summary:

I investigated the role of testosterone on olfactory processing in the common goldfish. Traditionally, steroid hormones like testosterone were believed to work genomically, by moving through cell membranes and affecting gene transcription. However, testosterone can also work rapidly by binding to membrane-bound receptors, which can cause rapid changes in behavior.

These rapid modulations likely come into play naturally in the context of mating, because it is a relatively quick process. In goldfish mating, female goldfish release the pre-ovulatory pheromone 12 hours prior to ovulation. In response, male goldfish produce a spike in testosterone. During ovulation, females release prostaglandin F2α and its metabolites, most notably, 15-keto prostaglandin F2α, both of which increase courtship behavior in male goldfish.

Previous research suggests that these rapid increases in testosterone may affect the olfactory processing of a goldfish. Male goldfish spend significantly more time near a pump releasing large volumes of PGF2α, the ovulatory pheromone, as compared to a pump releasing ethanol (unpublished data, Massa, 2014). Additionally, goldfish injected with testosterone just prior to testing spent more time near the source of the pheromone than did fish injected with a vehicle.

This summer, I sought to study the fishes' behavioral response to an attractive olfactory stimulus delivered to a tank using a pump that released pheromone at a rate that is similar to the rate at which female goldfish release 15-keto PGF2α. In order to test this new pump paradigm, I used a cue that is extremely potent, an amino acid food cue in the form of l-arginine. In this experiment, goldfish were isolated in a tank and their behavior recorded using Limelight tracking software. The fish habituated for 20 minutes, and then water was infused into the tank using the pump for 20 minutes, immediately followed by the amino acid. In this experiment, the fish spent significantly more time in the quadrant of the tank containing the pump when it was releasing l-arginine as compared to the water control ($p = 0.03$). In a second experiment, male goldfish were injected with ovaprim, a solution which sexually primes the fish, and then exposed them to ethanol, followed by 15-keto PGF2α. The fish spent significantly more time near the source of the pheromone as compared to the control ($p=0.04$). These two experiments validated the efficacy of the new pump system, and allowed me to proceed to studying the effects of testosterone and fadrozole on this behavior.

In the brain, testosterone either remains testosterone or is aromatized into estradiol. Fadrozole is a drug that blocks aromatase and therefore prevents testosterone from being converted to estradiol. This summer, I used the novel pump system to test the goldfishes' attraction to the pheromone after being injected with T, T + FAD, and a vehicle. However, I studied this attraction in non-milting, non-reproductive males. These males showed no preference for the pheromone, even when treated with T or T + FAD ($p=0.87$). This is unsurprising, as research conducted outside of the breeding season typically yields inconclusive results. I intend to rerun this experiment in the spring when the fish are milting and in the breeding season.

In addition to these behavioral studies, I also intend to study neural activity using immunohistochemistry. I collected brains from goldfish that were isolated and put into water with 15-keto prostaglandin F2α, l-arginine, and an ethanol control. By staining these brains with indicators of cell activity, pERK and ps6, I will be able to detect differences in cell activity between fish treated with pheromone, amino acid, and control. This will allow me to detect the neural pathways associated with olfaction. Additionally, the brains from the behavior experiment will allow me to compare the activity of brains of fish treated with T, T + FAD and control, to determine how testosterone may alter olfactory processing.

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