*Experimental Design:* Two milting fish (ranging ±5 g from each other) from a 160gal

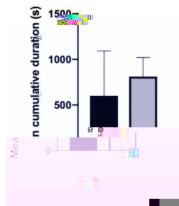
deviated from their natural behavior due to the stress caused by gluing on fluorescent hats and other handling in the experiment. The behavioral paradigm developed will allow for future experiments, perhaps with larger sample sizes, to track and observe the competitive behavior of male goldfish.

Determining an effective double-labeling immunohistochemistry protocol with PS6 and Anti-HuC will allow for an accurate method of counting the double-labeled colored cells per green cell for the purpose of obtaining a measure of activated neurons per total neurons in an area. With this protocol developed, we will be in position to identify whether testosterone induced more cell activity in response to sex pheromones and if so, where in the brain.

<u>Figures</u>



*Figure 1:* The mean distance from the male fish to the female fish in male subjects that ate a control pellet (n=11) and subjects that ate a T pellet (n=11). Distance was measured from the fluorescent hat on the male fish to the fluorescent hat on the female fish. Mean distance did not significantly decrease in the T pellet group (t-test, p>0.05).



*Figure 2:* The mean cumulative duration that the male fish was within proximity (5 cm) of the female fish (n=11). The cumulative duration of fish that had eaten a testosteron pellet did increase but was not significant (t-test, p>0.05).

(A)

(B)

(C)

*Figure 3:* Brain sections (20x magnification) at the preoptic area with an immunohistochemistry run with varying Anti-HuC dilutions. Midline of the brain is middle of image with no signal. (A) 1:500 dilution of anti-HuC. (B) 1:1000 dilution of anti-HuC. (C) 1:2000 dilution of anti-HuC.

*Figure 4:* Multifluorescence of midbrain at 20x magnification. Red signal (Alexa 594) is PS6 and green signal (Alexa 488) is Anti-HuC. An overlap indicates a neuron activated by sensory stimuli.

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