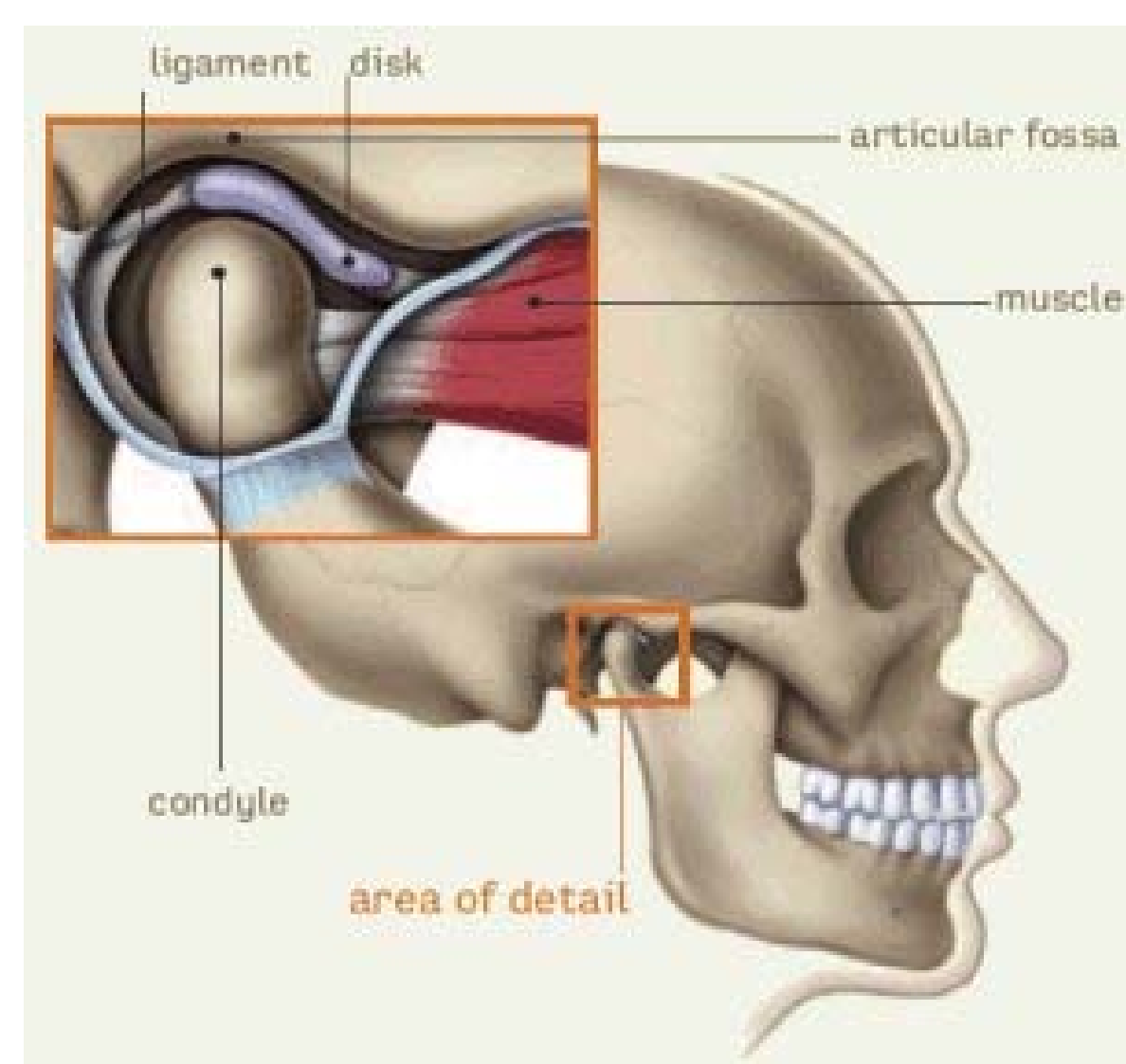


# The Effects of Estrogen and Stress on the Expression of Serotonin Receptors in the Rat Brainstem

Ashley Hillstrom (Randy Thompson and Dr. David Bereiter): Department of Diagnostic and Biological Sciences

## What is Temporomandibular Joint Disorder (TMJD)?

- TMJD's are a collection of painful conditions that involve the TMJ and muscles of mastication
- It affects between 5% and 15% of people in the US
- Symptoms include: pain/tenderness of jaw, aching facial pain, locking of the jaw, difficulty/discomfort while chewing



## Why look at estrogen and stress?

- Women in their childbearing years are more likely than men to develop TMJD
- There is a variation in pain severity over the menstrual cycle
- Women are more likely than men to develop anxiety/depressive illnesses
- Women with mood disorders are twice as likely to develop painful TMJD
- Serotonin is neurotransmitter in the brain linked to neural pathways involved in both pain sensation and mood disorders.
- Serotonin receptors are found in brain areas known to receive inputs from sensory nerves that innervate the TMJ region

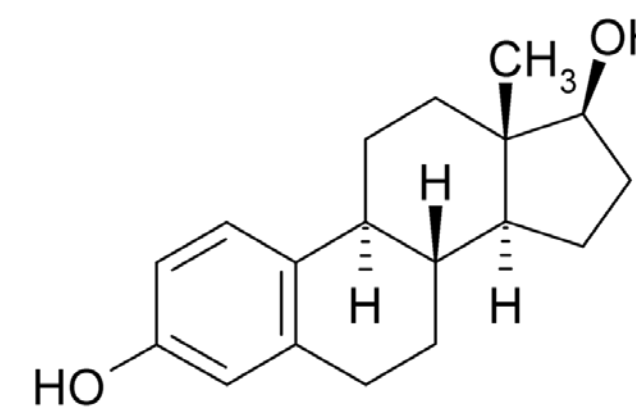
## Hypothesis

Does estrogen status and/or chronic stress alter the expression of serotonin receptors in regions of the brain known to be important for TMJ pain processing?

## Procedure

1. Start with ovariectomized (OvX) female rats

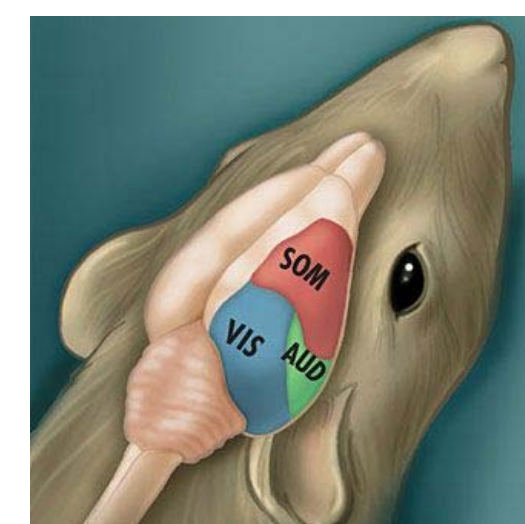
2. Inject with high or low levels of estradiol



3. Perform or not perform Forced Swim Test (FST) for ten minutes per day for three consecutive days to simulate chronic stress



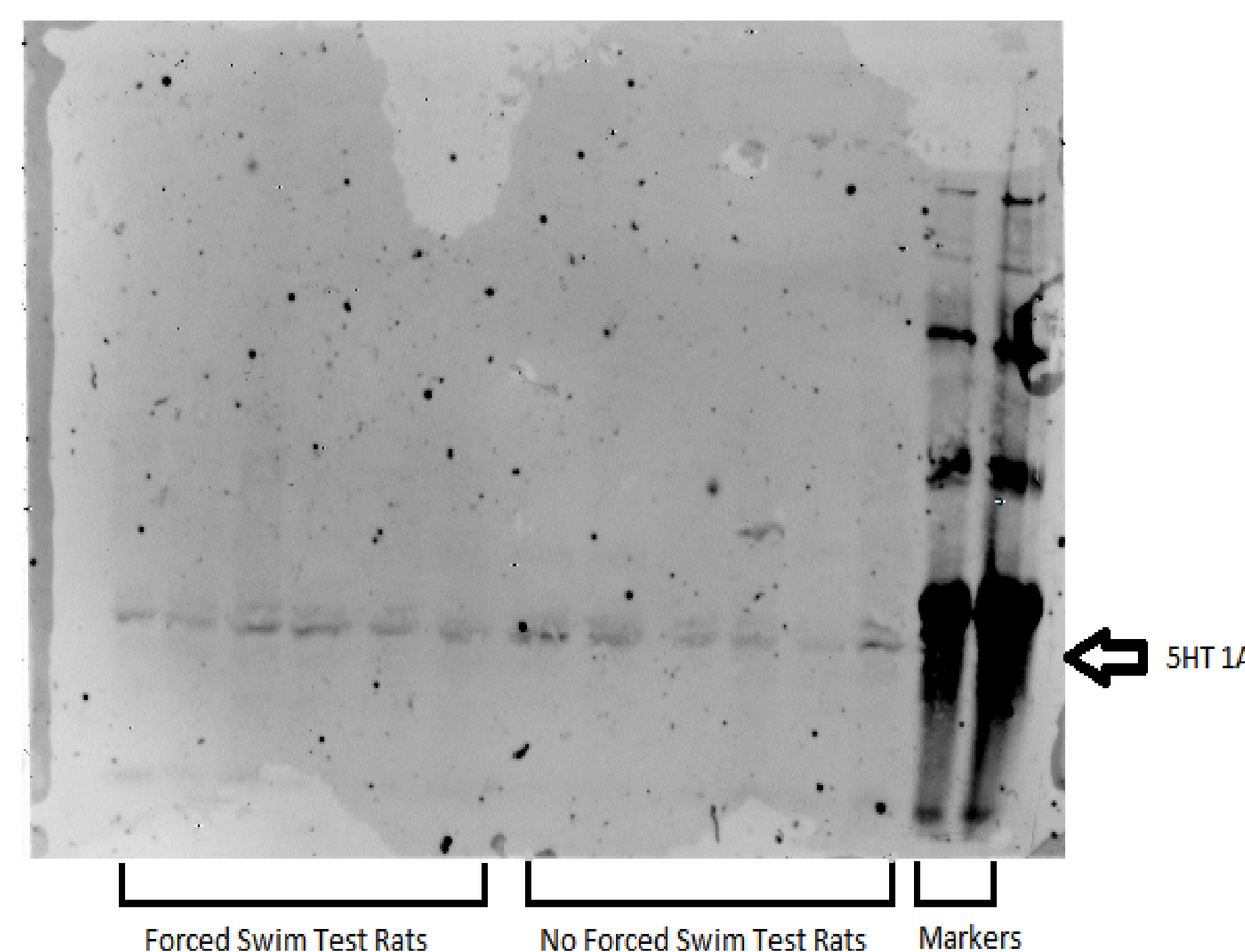
4. Sacrifice rat, dissect brainstem, and keep regions of the brain known to receive painful input from the TMJ



5. Process brainstem for level of serotonin receptors 5HT-1A, 5HT-2A, 5HT-3A, 5HT-4, and 5HT-7 by immunoblotting and quantitative polymerase chain reaction (qPCR)

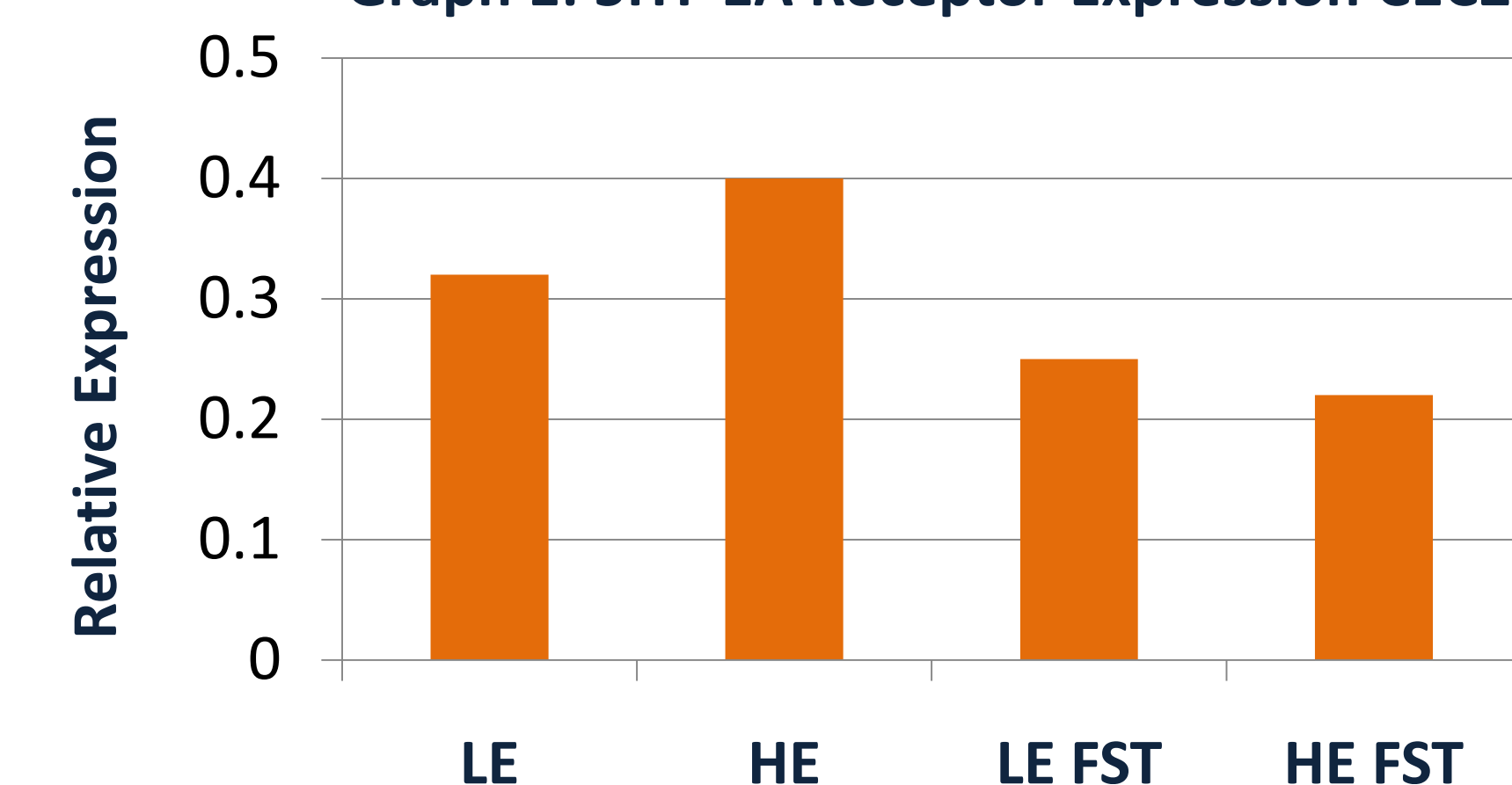
## Results from Western Blot

The western blot results were non-conclusive.



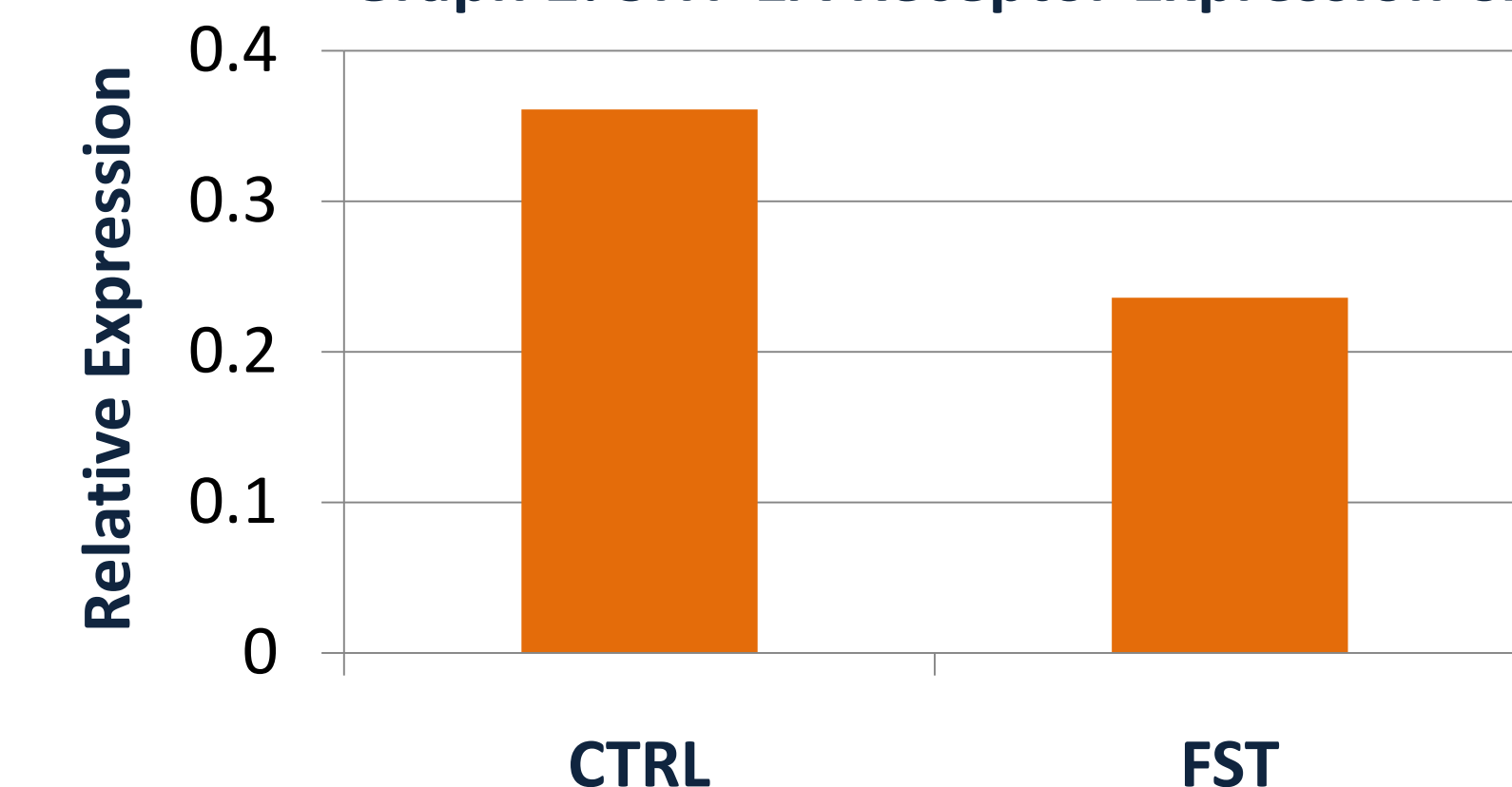
## Results from qPCR

Graph 1: 5HT-1A Receptor Expression C1C2



Of the five types of serotonin receptors studied, the qPCR results showed no statistically significant difference between high (HE) and low (LE) estrogen groups (example shown in Graph 1). However, the qPCR did show that for receptor 5HT-1A, there is a statistically significant difference between FST and control groups (Graph 2).

Graph 2: 5HT-1A Receptor Expression C1C2



For receptor 5HT-1A, although estrogen status does not appear to play a role in receptor expression, chronic swim stress does. After being subjected to FST there is a down-regulation of the receptor 5HT-1A as compared the control. This shows that chronic stress does alter the expression of serotonin receptors in the C<sub>1</sub>C<sub>2</sub> region of the brain known to be important for TMJ pain processing. Since receptor 5HT-1A is known to play a role in relieving anxiety and depression, the decreased expression level in FST rats shows that the rats are likely experiencing more anxiety than the control rats.

## Acknowledgements and References

I would like to thank David Bereiter for the opportunity to work on this research project. I would also like to thank Randy Thompson for all of the help and guidance throughout my experiments. Finally, I want to thank the University of Minnesota Undergraduate Research Opportunity Program for funding this project.

[http://www.aaoms.org/images/article\\_images/tmj/tmj\\_anatomy.jpg](http://www.aaoms.org/images/article_images/tmj/tmj_anatomy.jpg)  
<http://upload.wikimedia.org/wikipedia/commons/thumb/1/15/Estradiol.png/800px-Estradiol.png>  
[http://all-free-download.com/images/graphicmedium/purity\\_water\\_wave\\_21948.jpg](http://all-free-download.com/images/graphicmedium/purity_water_wave_21948.jpg)  
[http://www.monochrom.at/english/pictures/rat\\_brain1.jpg](http://www.monochrom.at/english/pictures/rat_brain1.jpg)

Bereiter DA, Cioffi JL, et al. Local blockade of integrins in the temporomandibular joint region reduces Fos-positive neurons in trigeminal subnucleus caudalis of female rats produced by jaw movement. *Pain*. 2006 Nov;125(1-2):65-73. Epub 2006 Jun 9.

Duenes SL, Thompson R, Chang Z, Okamoto K, Bereiter DA. Psychophysical stress increases the expression of phospho-CREB, Fos protein and neurokinin-1 receptors in superficial laminae of trigeminal subnucleus caudalis in female rats. *Neurosci Lett*. 2010 Dec 17;486(3):207-10. Epub 2010 Sep 25. PubMed PMID: 20884322; PubMed Central PMCID: PMC2967612.