

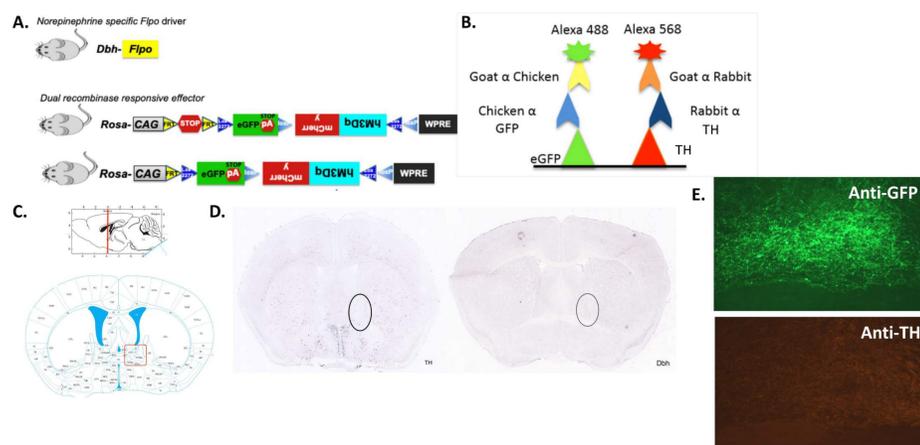
Overview

In this experiment, we studied tyrosine hydroxylase (TH) expression in the bed nucleus of the stria terminalis (BNST), focusing on possible sex differences. Tyrosine hydroxylase was chosen because of its implications in norepinephrine modulation, and the focus on the BNST is due to its role in stress responses. We chose to look at the TH expression across sexes because female are more likely than male to be diagnosed with stress related psychopathologies (Bangasser et al., 2016).

Hypothesis

TH expression in the BNST of female mice will be greater than the TH expression in the BNST of male mice.

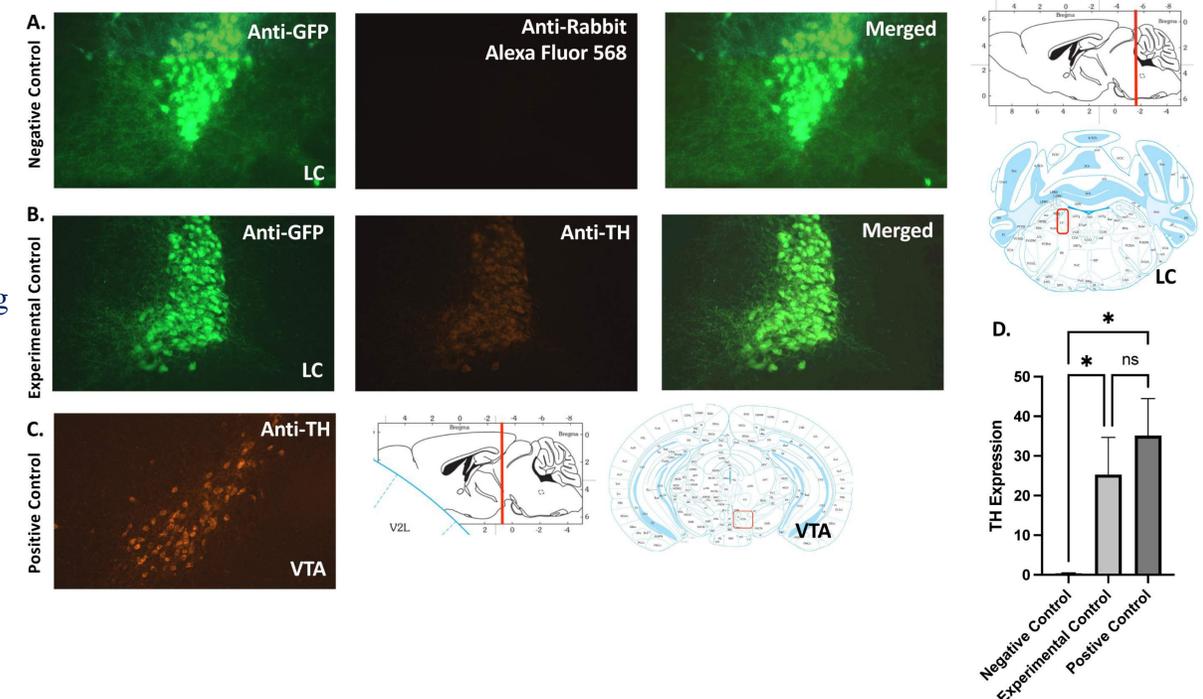
Methods



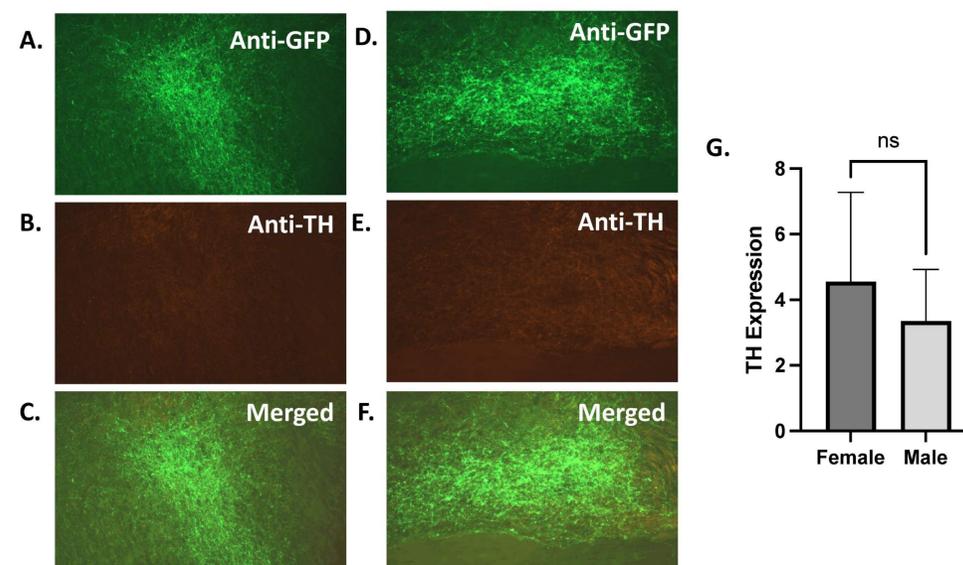
- Immunohistochemistry was used to stain all NE neurons and all neurons containing the TH enzyme.
- NE neurons expressing GFP (green) were labeled using an anti-GFP antibody Alexa 488
- Neurons expressing TH (red) were then labeled using a TH anti-body
- Brain sections were imaged on a Nikon ECLIPSE Ts2 Microscope using the Nikon NIS-Elements software. Brain regions were identified using the Allen Mouse Brain Atlas and imaged at 20x magnification at 470 nm for 700 ms for green NE expression and 560 nm for 800 ms for red TH expression.
- TH expression was quantified as mean pixel intensity per area relative to the background with FIJI software.

Results

- Coronal section from an adult mouse hindbrain and forebrain reveals successful staining of norepinephrine neurons and TH enzyme through immunohistochemistry protocol.
- The schematic diagram (right) represents the corresponding location of the representative sections (left).
- Negative control: LC of a mouse brain that was not treated with TH anti-body shows a successful staining of GFP in left image, and no red background in the middle showing no TH stain.
- Experimental control: LC of a mouse brain shows a successful staining of both stains GFP and TH.
- Positive Control: Imaged the VTA of a mouse brain was used because the VTA is known to have DA nuclei which express TH. Shows successful TH staining.
- Negative control have significantly lower TH expression that both the experimental and positive controls $p=0.0309$ and $p=0.0120$ respectively



Results



- Panels A-C are images taken from a female mouse BNST
- Panels D-F are images taken from a male mouse BNST
- No significant difference in TH expression between female and male BNST, $p=0.3558$

Conclusion and Future Direction

- **There is no significant difference in TH expression in the BNST between male and female mice.**
- Further experimentation with a larger sample size would allow us to be more confident in our findings.
- Since it is known that the BNST is involved in regulating stress further experimentation on sex differences in this brain region may give better insight into the discrepancies seen in prevalence of stress related disorders in females compared to males

Acknowledgements and Citations

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Bangasser, Debra A., Kimberly R. Wiersielis, and Sabina Khantsis. "Sex Differences in the Locus Coeruleus-Norepinephrine System and Its Regulation by Stress." *Brain Research, New Evidence for Heterogeneous Organization and Actions of the Central Noradrenergic Transmitter System*, 1641 (June 15, 2016): 177-88. <https://doi.org/10.1016/j.brainres.2015.11.021>.