



# Sex Differences in Tyrosine Hydroxylase Expression in Norepinephrine Neuron Subpopulations Sub CD/CV in Mice

Ben Garside, Valerie Muzyka, Manav Patel, Adriana Schott, Sabrina Robertson, PhD

## Introduction

The norepinephrine (NE) system is known to modulate stress response, commonly known as “fight or flight”, as well as mood, arousal, learning and memory.

Tyrosine hydroxylase (TH), our gene of interest (GOI), is found throughout the entire NE system, expressing similarly to dopamine beta hydroxylase (DBH), which allows the visualization of neurons within the NE system.

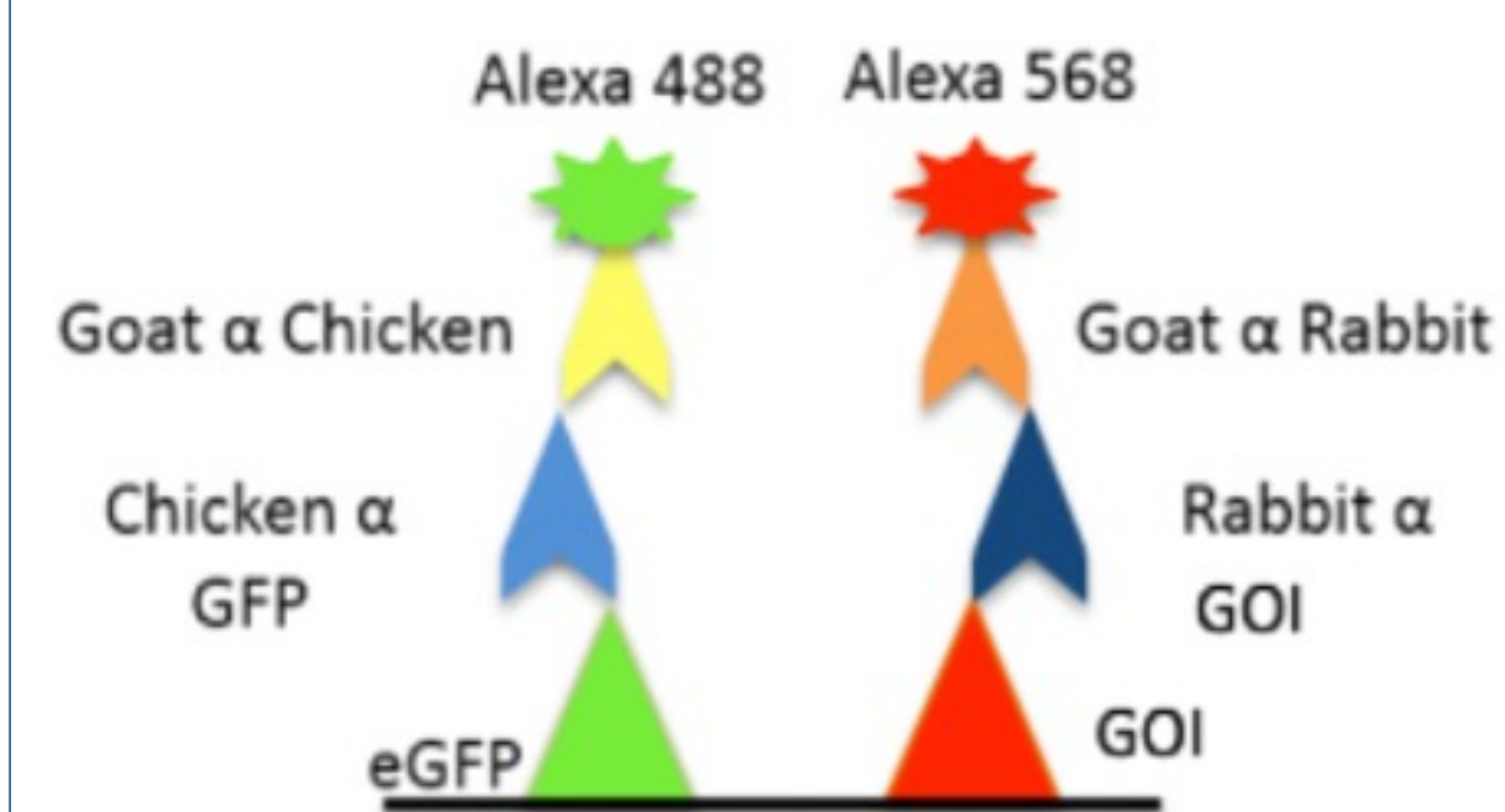
Previous research has investigated the modulation of TH in Alzheimer’s Disease (AD) and Parkinson’s Disease (PD) patients that occurs as a result NE neuron loss, but little research has focused on location of TH expression itself.

Further exploration of the location and sex differences of TH expression in the Sub CD/CV could lead to novel discoveries regarding sex-specific predisposition for neurodegenerative disease as well as possible preventative measures.

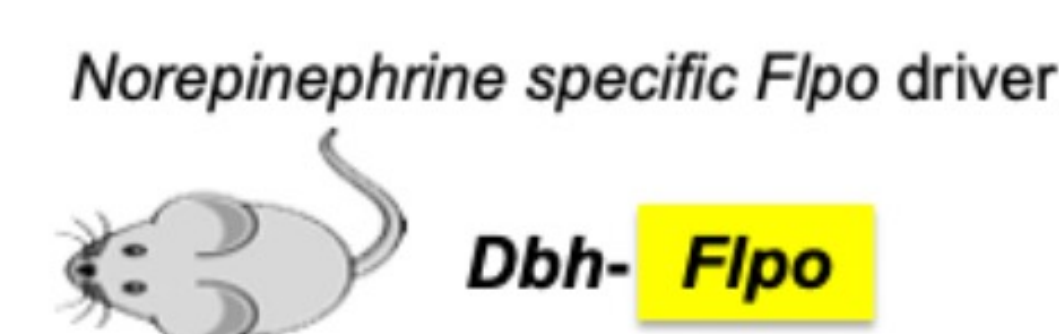
## Hypothesis

Evaluation of TH expression in the norepinephrine system is expected to exhibit observable differences in TH expression in norepinephrine neurons of the Sub CD/CV between male mice and female mice.

## Methods



DBH-Flpo transgenic mice were used as our subjects. N = 10 for both male and female specimens respectively. Dual- antibody immunohistochemistry (left) allowed us to identify NE neurons and TH expression.



After IHC, a Nikon ECLIPSE Ts2 microscope was utilized to image GFP and TH expression. Merged images were developed to visualize colocalization of GFP and TH within NE neurons in the Sub CD/CV.

Dual recombinase responsive effector



## Results

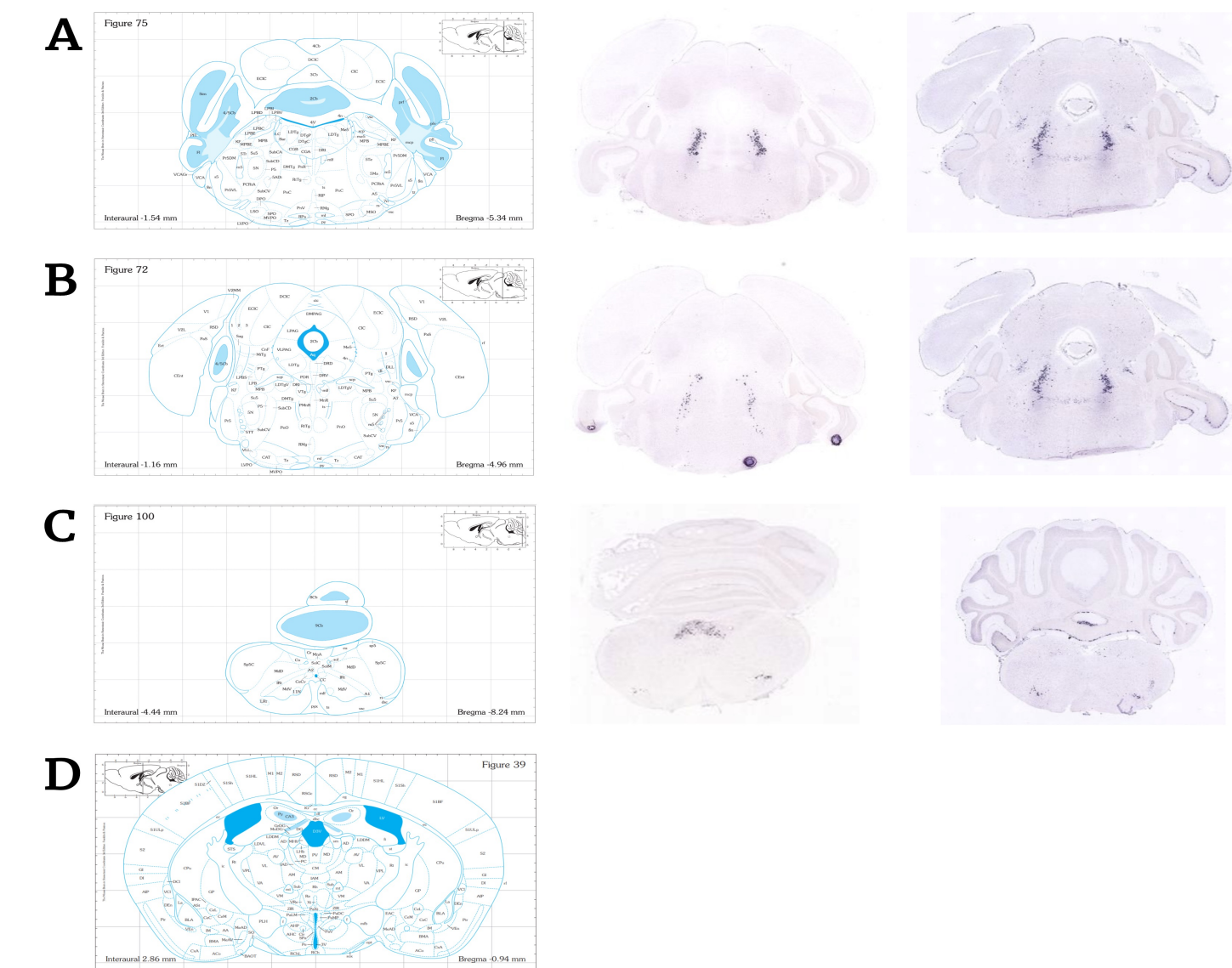


Figure 1. Comparison of dopamine beta-hydroxylase (DBH) and tyrosine hydroxylase (TH) expression in different anatomical subregions of the norepinephrine system. Dbh (middle) and TH (right) expression stained on coronal sections of the mouse hindbrain (A, B, C) and forebrain (D), corresponding to specific anatomical subregions (left). Regions of interest include LC (A), SubCD and SubCV (B), A1 and A2 (C), and the amygdala (D). Anatomical maps were collected from the Franklin and Paxinos Mouse Brain Atlas and expression data was collected from the Allen Mouse Brain Atlas.

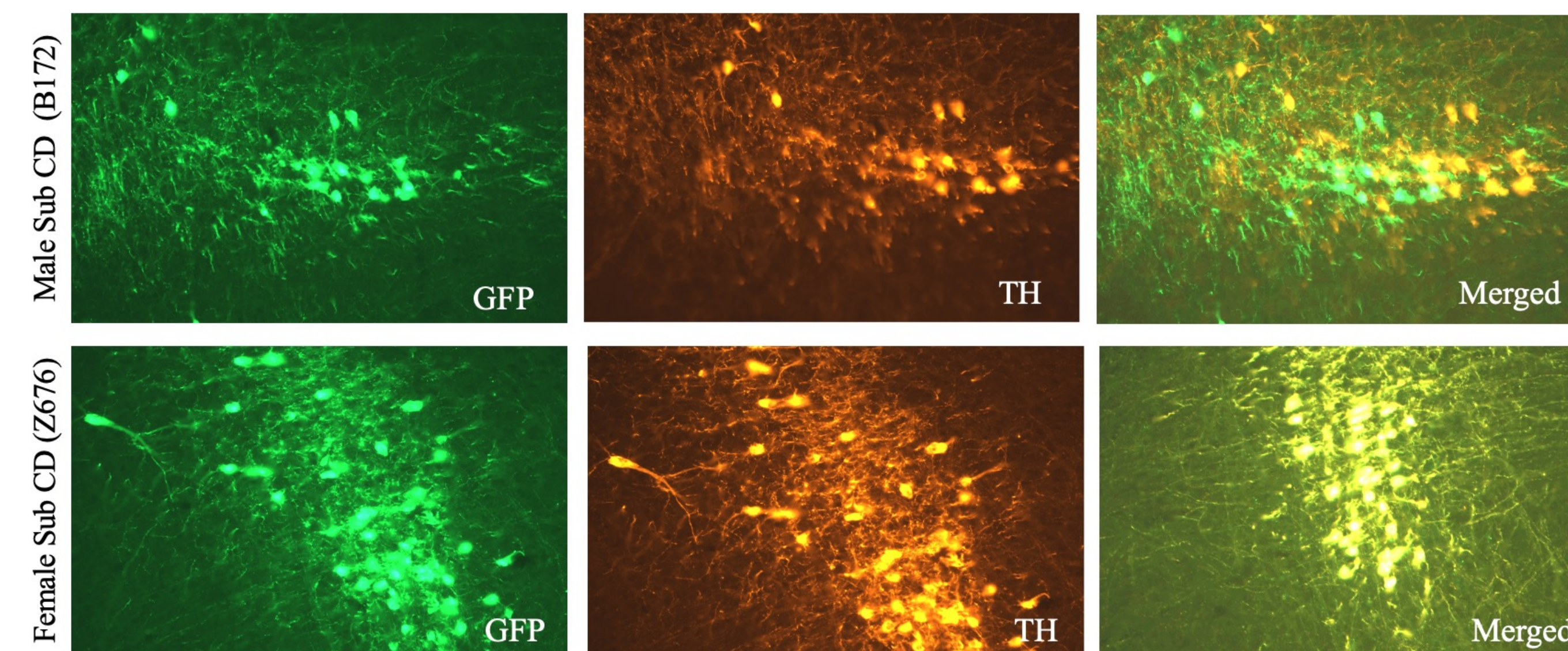


Figure 4. Comparison of NE neurons and TH expression in Sub CD between male and female mice. Images in top row reflect expression of GFP (left), TH (middle) and both GFP and TH (right) in Sub CD of a male mouse. Images in the bottom row reflect expression of GFP (left), TH (middle) and both GFP and TH (right) in Sub CD of a female mouse.

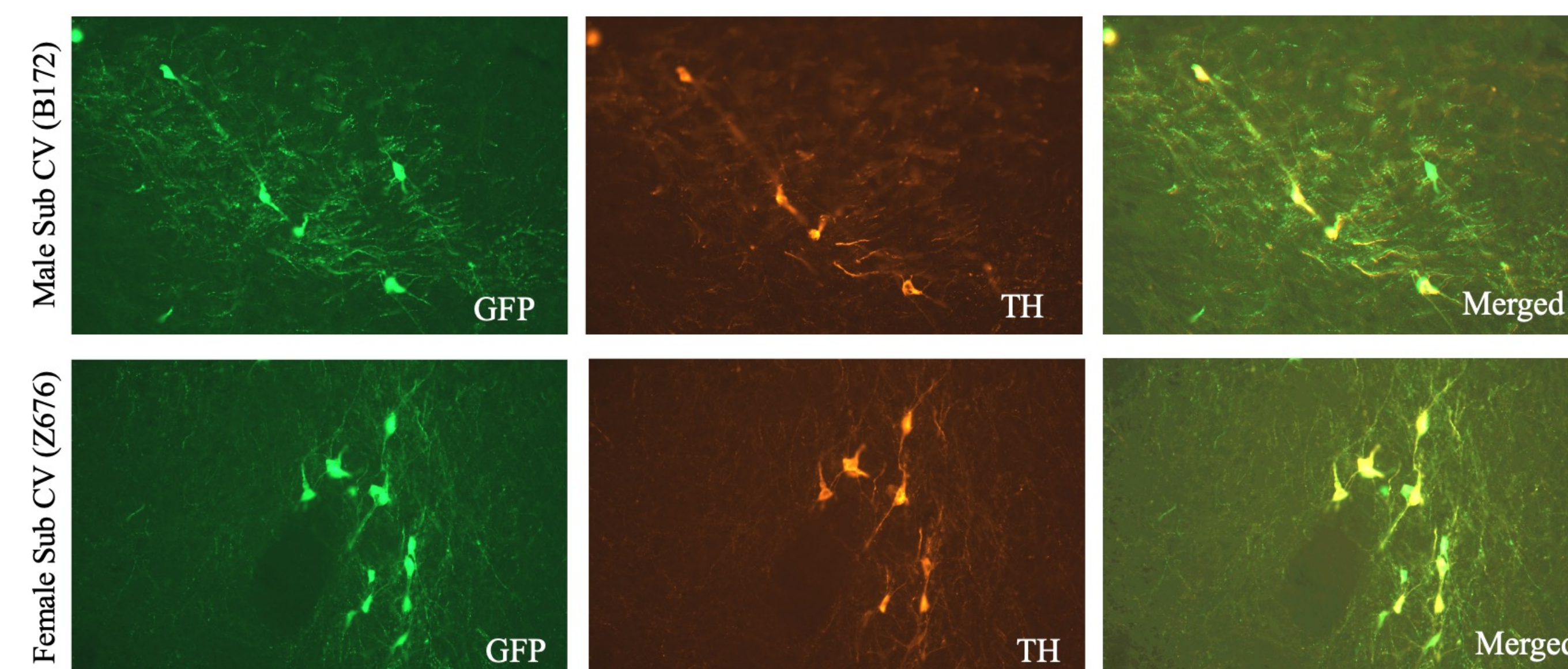


Figure 5. Comparison of NE neurons and TH expression in Sub CV between male and female mice. Images in top row reflect expression of GFP (left), TH (middle) and both GFP and TH (right) in Sub CV of a male mouse. Images in the bottom row reflect expression of GFP (left), TH (middle) and both GFP and TH (right) in Sub CV of a female mouse.

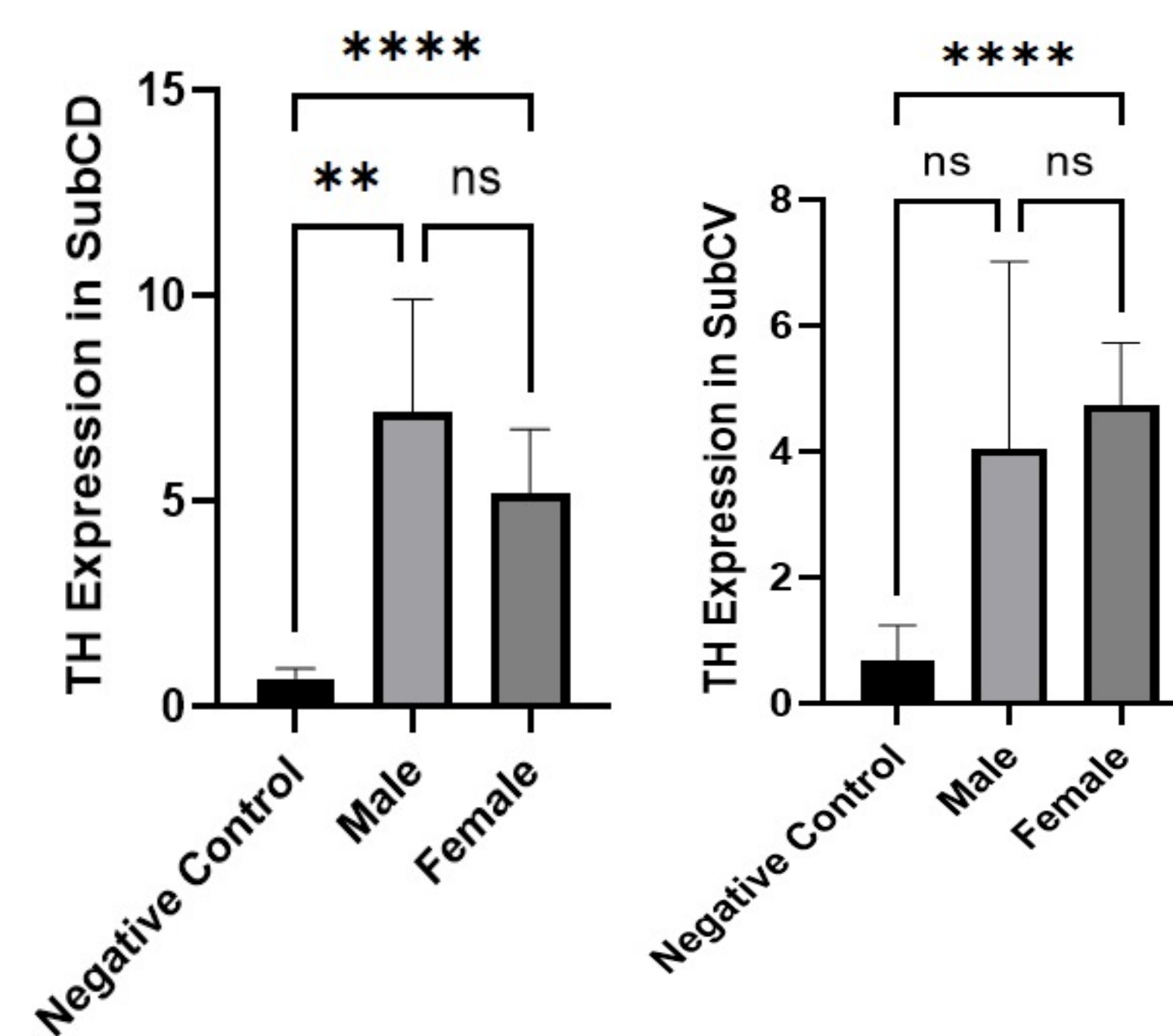


Figure 6. Evaluation of TH expression between male and female mice in Sub CD/CV. Welch’s one-way ANOVA test shows that both male and female mice had significantly greater TH expression per unit area compared to the control group in the Sub CD region (left). No significant differences between the male and female groups in this region were observed. Both male and female mice had significantly greater expression of TH per unit area compared to the control group in the Sub CV region (right). Significant differences between male and female groups were not observed.

(“\*\*”) indicates p > 0.01 and (“\*\*\*\*”) represents p > 0.0001.)

## Discussion

Using a Welch’s one-way ANOVA test, it was determined that statistically significant differences in TH expression within the Sub CD were not found with respect to sex. Males, p = 0.0017, and females, p < 0.0001, both had significantly greater expression of TH compared to the control group, but not compared to each other, p > 0.05. While not a statistically significant difference, expression was found to be greater overall in male subjects.

A similar statistical approach was conducted within the Sub CV. The test, once again, revealed no statistically significant differences in TH expression with respect to sex. Females, p < 0.0001, had significantly greater expression of TH compared to the control group, but males, p > 0.05, did not. Males and females also did not have significantly different expression of TH, p > 0.05, though, expression was found to be greater overall in female subjects.

Based on these results, we reject our initial hypothesis since no statistically significant differences in expression were found in either NE neuron subpopulation. This suggests that TH expression would not be successful as a predeterminant for the development of neurodegenerative diseases, such as AD and PD, with respect to sex.

## Limitations and Future Directions

- Expression levels of TH were only analyzed within two NE neuron subpopulations – Sub CD/CV
- A larger sample size should be studied to make the research more comprehensive, impactful and representative of the population of transgenic mice studied
- Different dilutions of TH were utilized amongst research groups when creating the dual antibody solution
- Future directions may consider phases of menstrual cycle as they have not been previously considered or standardized across female subjects

## References

### Acknowledgements

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