

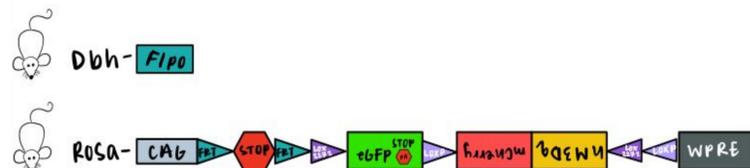
# Sexual Dimorphism of Norepinephrine Neuron Projections and Calcitonin Gene-Related Peptide Expression within the BNST

## Overview

- NE neurons interact with CGRP, both affecting general stress and anxiety responses.
- Both CGRP and NE neurons show sexually dimorphic physiology.
- NE neurons project to the BNST, but little research exists on potential CGRP activity in the BNST.
- We expect: **greater CGRP expression within the BNST in females compared to males.**

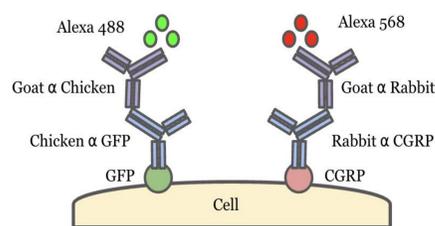
## Methods

Mice brain tissues are genetically engineered to have NE neurons express enhanced green fluorescent protein (eGFP).



**Figure 1.** Schematic diagram illustrating the norepinephrine specific *Flpo* driver for *Dbh* (top) and the dual recombinant responsive effector (bottom).

IHC methods were used, employing primary and secondary antibodies that helped to illuminate eGFP and CGRP expressing neurons during imaging.



**Figure 2.** Dual primary antibody and secondary antibody protocol allow for identification of eGFP- and CGRP- expressing neurons

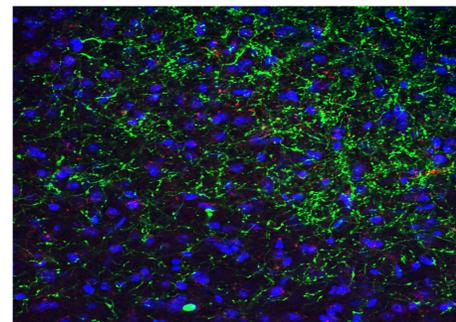
Imaging analysis protocols conducted after imaging allowed for visualization of CGRP and eGFP expression. Further analyses were possible that quantified expression.

**Table 1.** Primary and Secondary Antibodies and Their Properties

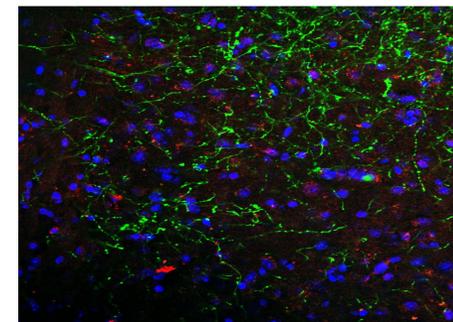
Antibody	Host Species	Dilution	Manufacturer, Catalog #	
<b>Primary Antibodies</b>	Chicken anti-GFP	Chicken	1:10000	Abcam, AB13970
	Rabbit anti-CGRP	Rabbit	1:2000	Abcam, AB47027
<b>Secondary Antibodies</b>	Goat anti-chicken Alexa 488	Goat	1:1000	Abcam, AB150169
	Goat anti-rabbit Alexa 568	Goat	1:1000	Abcam, AB175471

Alvin Dinh, Bryson Curtis, Kathy Nguyen, Zander Pittman  
Sabrina Robertson, PhD (Advisor)

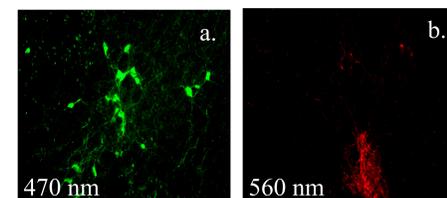
## Results



**Figure 3.** Confocal merged images of male BNST. Staining for nuclei (blue), NE neurons (green), and CGRP (red)



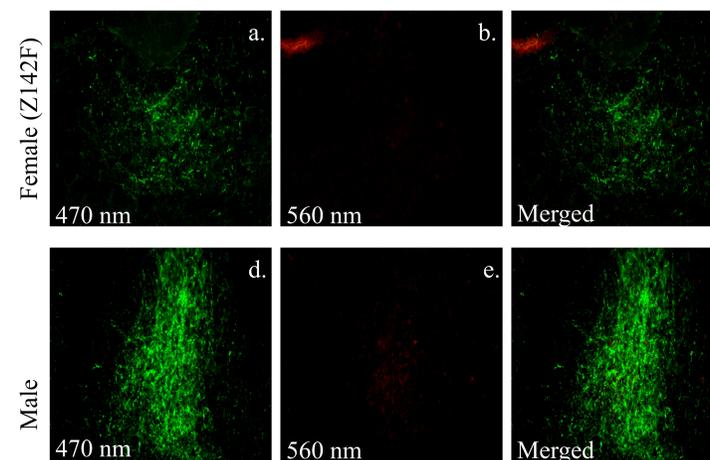
**Figure 4.** Additional confocal merged images of male BNST. Staining for nuclei (blue), NE neurons (green), and CGRP (red)



**Figure 5.** Immunofluorescent eGFP (left) and CGRP (right) expression in A1 region from animal C111, male

Images produced show CGRP and eGFP immunoreactivity as indicated by red (CGRP) and green (eGFP) fluorescence. The presence of immunoreactive CGRP and eGFP-expressing NE neurons (Figures 3, 4, 5) demonstrate conducted immunohistochemistry protocols were successful.

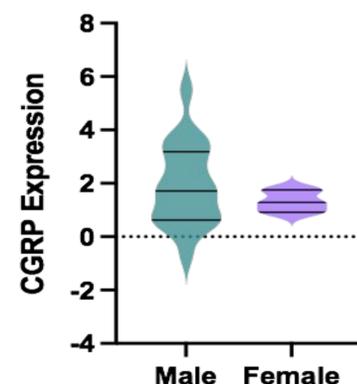
- CGRP is scarce in both male and female BNST.
- Male BNST has denser eGFP-expressing NE projections than female BNST.
- No colocalization of CGRP and NE neurons.



**Figure 6.** Immunofluorescent imaging of CGRP and *Dbh* within the BNST of mice Z142F and C111M

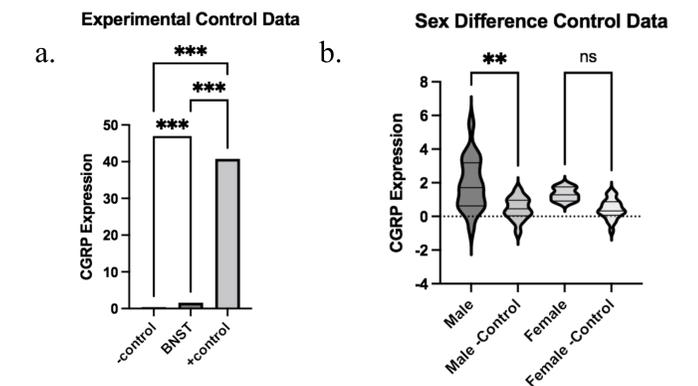
Results of the Welch's t-test indicate that there is no significant difference in CGRP expression within the BNST between male ( $M = 1.93$ ,  $SD = 1.54$ ) and female ( $M = 1.31$ ,  $SD = 0.42$ ) mice,  $t = 1.40$ ,  $p = 0.185$ . These results contradict our initial hypothesis that female CGRP expression would be higher and indicate that CGRP expression levels in the BNST may not play a significant role in mediating sex differential stress responses in mice.

## Mice Data



**Figure 7.** Relative CGRP expression between male and female mice

## Controls



**Figure 8.** Study controls used to validate imaging analysis approach and sex differences in CGRP expression

From the experimental control (a.), it is evident that our samples were successfully stained for CGRP and that CGRP was present in the BNST. The sex difference control (b.) indicates that while CGRP expression in male mice was significant, the female BNST showed no expression above background levels.

## Conclusion

- Sex differentiation of eGFP-expressing projections was found; projections in male BNST denser than female BNST.
- Little CGRP expression was found for both sexes, unable to discern possible sex differences in BNST.
- CGRP may not be heavily linked to the BNST or any responses mediated by this region.
- Future studies should utilize a larger sample size ( $n=2$ ).

## Limitations

- Shortened research period requiring generalization of results.
- Limited viable female BNST samples.

## Acknowledgments

We would like to thank Dr. Sabrina Robertson, Janay Franklin, Cami Arzt, and Emma Pfeiler for their support and NIEHS for their donation of mice brains to make this research possible.