Calcitonin gene-related peptide (CGRP) exists as a peptide vasodilator and is believed to play a significant role in migraine, hypertension, and appetite. The Locus Coeruleus (LC) and dorsal Subcoeruleus (SubCD) nuclei are two key subpopulations of the noradrenergic system, which regulates cardiovascular, respiratory, and visceral aspects of hyperarousal. Previous neuroscience research exploring sex differences in migraine revealed that female mice had higher levels of mRNA for the gene encoding CGRP than their male counterparts. CGRP has been implicated in migraine, a neurovascular disease that disproportionately affects the female population. Research exploring sex differences in the noradrenergic system is lacking due to historic sex bias in neuroscience research. There also exists a lack of research investigating the presence of CGRP in the noradrenergic system. Disruption of the norepinephrine system is associated with a variety of neurodegenerative disorders, many of which are also present more often in females. Therefore, it is imperative that we study the overlap of these two systems to develop a greater understanding of their relationship and the potential therapeutics applications for these disorders.

**Methods**

- Transgenic mice expressing enhanced Green Fluorescent Protein (eGFP) in NE neurons
- Primary antibodies applied to coronally cryosectioned mouse brain tissue in order to tag GFP labeled neurons and CGRP
- Secondary antibodies containing fluorophores used to tag the primary antibodies

**Figure 1.** Schematic representation of coronal cryosectioned mouse brain tissue in order to tag GFP labeled neurons and CGRP

**Figure 2.** Schematic representation of antibody binding

**Background**

**Objectives**

1. We expect to see colocalization of CGRP in the Locus Coeruleus and Dorsal Subcoeruleus NE subpopulations.
2. We expect to see increased expression of CGRP in female mice compared to their male counterparts.

**Hypothesis**

1. The first hypothesis was proven correct by the Epifluorescence images of NE and CGRP expressing neurons in the LC & SubCD regions. The Epifluorescence images overlayed NE and CGRP expressing neurons show co-localization and co-expression. (Fig. 4 and Fig. 5)
2. Our second hypothesis was incorrect and no statistically significant differences were seen in the co-expression of NE and CGRP in the LC. While this data suggests that co-expression may be present, a more standard method of quantification should be applied to this observation in order to better determine significance. Future research is needed to further analyze evidence of co-expression and uncover the nature of the relationship between NE and CGRP expressing neurons in other subpopulations in order to further investigate the root of differential prevalence of conditions like migraines amongst sexes.

**Results**

**Figure 4: SubCD**

(a) Fluoform images of NE and CGRP expressing neurons with red overlay in the SubCD subpopulation. (b) Overlay image of each expressing neuron at 40x magnification – Mouse: B177F. (c) Epifluorescence images of (a) NE and (b) CGRP expressing neurons with (c) overlay in the SubCD subpopulation. (d) Overlay image of each expressing neuron at 40x magnification – Mouse: B177F.

**Figure 5: LC**

(a) Fluoform images of NE and CGRP expressing neurons with red overlay in the LC subpopulation. (b) Epifluorescence images of (a) NE and (b) CGRP expressing neurons with (c) overlay in the LC subpopulation. (d) Overlay image of each expressing neuron at 40x magnification – Mouse: B177F.

**Future Directions**

- Future research is needed to further analyze evidence of co-expression and uncover the nature of the relationship between NE and CGRP expressing neurons in the LC and SubCD
- While this data suggests that co-expression may be present, a more standard method of quantification should be applied to this observation in order to better determine significance
- Exploration into sex differences in co-expression of NE and CGRP expressing neurons in other subpopulations is necessary to further investigate the root of differential prevalence of conditions like migraines amongst sexes.

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**References**

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**Conclusion**

- The first hypothesis was proven correct by the Epifluorescence images of LC and SubCD regions that overlaid NE and CGRP expressing neurons show co-localization and co-expression (Fig. 4 and Fig. 5).
- Average max fluorescence of co-expressed CGRP in the LC is significantly higher than that of the SubCD suggesting greater co-expression of NE and CGRP in the LC (Fig. 6).
- Our second hypothesis was incorrect and no statistically significant differences were seen in the co-expression of NE and CGRP in male versus female rats in the LC nor the SubCD (Fig. 7 and Fig. 8).

**Future Directions**

- Exploration into sex differences in co-expression of NE and CGRP expressing neurons in other subpopulations is necessary to further investigate the root of differential prevalence of conditions like migraines amongst sexes.