Exploration of CGRP co-expression in the LC and SubCD noradrenergic subpopulations of male and female mice

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Background

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 neuromodulatory norepinephrine (NE) system, which regulates the cardiovascular, respiratory, and visceral aspects of hyperarousal. Previous research investigating sex differences in migraine revealed that female mice had higher levels of mRNA for the gene encoding CGRP than their male counterparts[1]. CGRP has been implicated in migraine, a neurovascular disease that disproportionately affects the female population[2]. Research exploring sex differences in the norepinephrine system is lacking due to historic sex bias in neuroscience research[3]. 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 therapeutic applications for these disorders.

Objectives

We aim to define CGRP expression in the LC and SubCD noradrenergic subpopulations. We also hope to identify any sex differences that are present in CGRP expression in those NE subpopulations.

Hypothesis

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.

Methods

Immunohistochemistry

- 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

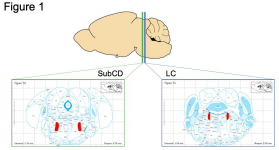

Figure 1. Schematic representation of coronal cryosectioning of mouse brain. *Images taken from Allen Mouse Brain Atlas*[4].

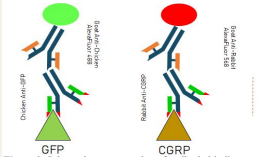

Figure 2. Schematic representation of antibody binding

Secondary Antibody

Primary Antibody

Microscopy

Captured images from the LC and the SubCD for analysis

Results

Figure 4: SubCD

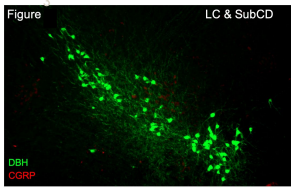

Figure 3. Epifluorescence image of NE and CGRP expressing neurons in the LC & SubCD regions. *Image taken at 10x magnification – Mouse: A020F.*

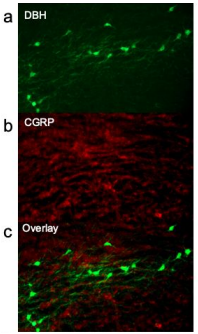

Figure 4. Epifluorescence images of **(a)** NE and **(b)** CGRP expressing neurons with **(c)** overlay in the SubCD subpopulation. *Epifluorescence images obtained at 20x magnification – Mouse: B177F.*

Figure 5: LC

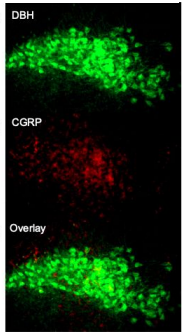

Figure 5. Epifluorescence images of **(a)** NE and **(b)** CGRP expressing neurons with **(c)** overlay in the LC subpopulation. *Epifluorescence images obtained at 20x magnification – Mouse: B177F.*

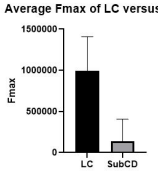

Figure 6. Average Fmax of LC versus SubCD. An unpaired t test showed the difference was statistically significance with a P value of 0.0010.

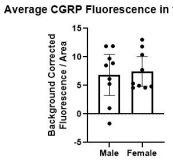

Figure 7. Average Background Corrected CGRP Fluorescence in Male vs Female Rats in the LC Region.3` An unpaired t test showed no statistical significance with a P value of 0.75.

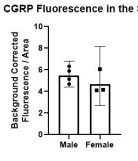

Figure 8. Average Background Corrected CGRP Fluorescence in Male vs Female Rats in the SubCD Region. An unpaired t test showed no statistical significance with a P value of 0.39.

Conclusion

- The first hypothesis was proven correct by the Epifluorescence images of LC and SubCD regions that overlayed 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

- 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

References

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2. Stucky, Nicholas L et al. "Sex differences in behavior and expression of CGRP-related genes in a rodent model of chronic migraine." *Headache.*
3. Will, Tyler R et al. "Problems and Progress regarding Sex Bias and Omission in Neuroscience Research." *eNeuro.*
4. Allen Mouse Brain Atlas
Lein, E.S. et al. (2007) Genome-wide atlas of gene expression in the adult mouse brain, Nature 445: 168-176. doi:10.1038/nature05453

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