Exploration of Molecular Sex Differences in CGRP-Expressing Noradrenergic Neurons in A1 and A2

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BACKGROUND

Calcitonin gene-related peptide (CGRP) is a peptide vasodilator known to be highly implicated in migraine pathogenesis, which is more prevalent in females than males (1). Recent studies have identified a genetically defined subpopulation expressing CGRP that plays a functional role in sex differentiation within the Locus Coeruleus (LC), a key nuclei of the norepinephrine system which regulates multiple diverse behaviors and psychological processes (2,3).

"MIGRAINES ARE TWICE AS PREVALENT IN WOMEN THAN MEN"

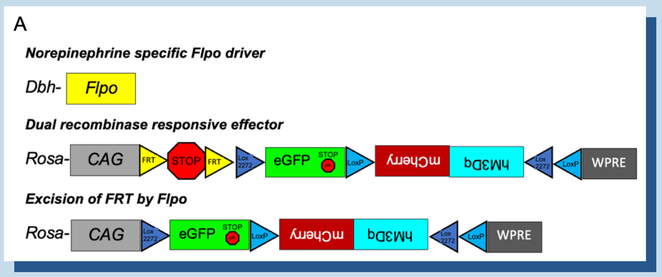
Although there is evidence of CGRP's sex-differential response in LC-NE neurons, there is limited research exploring CGRP's interaction within other NE subpopulations. Additionally, due to the historic sex bias and sex omission present in neuroscience literature, research surrounding sex differences within the NE system is also lacking. In this study, we aim to quantify CGRP expression in the A1 and A2 noradrenergic nuclei using a dual IHC method on a transgenic strain of female and male mice.

HYPOTHESIS

The expression of CGRP in the noradrenergic anatomical subpopulations of A1 and A2 will be higher in female mice as compared to male mice.

The norepinephrine system is involved with many common neurological disorders that are mediated by molecular sex differences in distinct neuronal populations; therefore, this study could contribute to the possible mechanisms of treatment for these disorders and broaden our understanding of the relationship between CGRP and the norepinephrine system.

METHODS



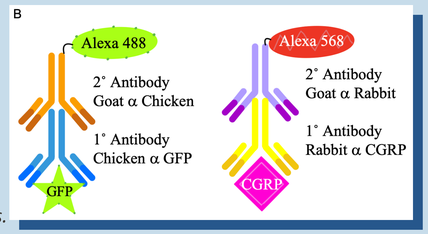


Figure 1
A. *Triple transgenic driver line to enable expression of GFP in all norepinephrine neurons*
B. *Schematic of dual immunohistochemistry of primary and secondary antibodies to amplify GFP and stain CGRP-expressing neurons.*

EXPERIMENTAL TIMELINE

A dual primary antibody solution was used to label CGRP and eGFP-expressing norepinephrine neurons → **A secondary antibody was used to label NE neurons to show green fluorescence and CGRP neurons to show red fluorescence** → **eGFP-expressing neurons analyzed under 470 nm light and mCherry-expressing neurons analyzed under 590 nm light**

RESULTS

After successful antibody staining, we see both immunoreactivity for eGFP-expressing NE neurons and CGRP neurons (Figure 2). Both eGFP-expressing NE neurons and CGRP neurons displayed immunoreactivity, and overlaid images of A1 and A2 highlight low co-expression of NE and CGRP in each subpopulation. Additionally, sex differential expression of CGRP was explored via image analysis. Figure 2 shows A1 and A2 fluorescence across the sexes.

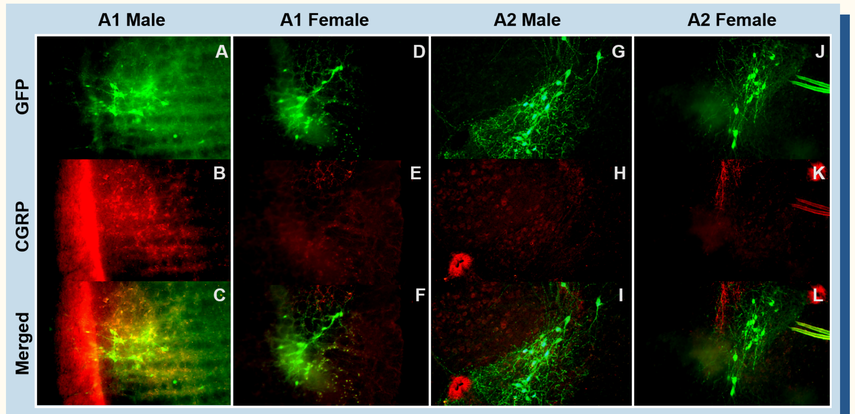


Figure 2. *Immunofluorescent imaging of GFP, CGRP and a merged image of A1 in male (A-C) and female (D-F) mice, and A2 in male (G-I) and female (J-L) mice.*

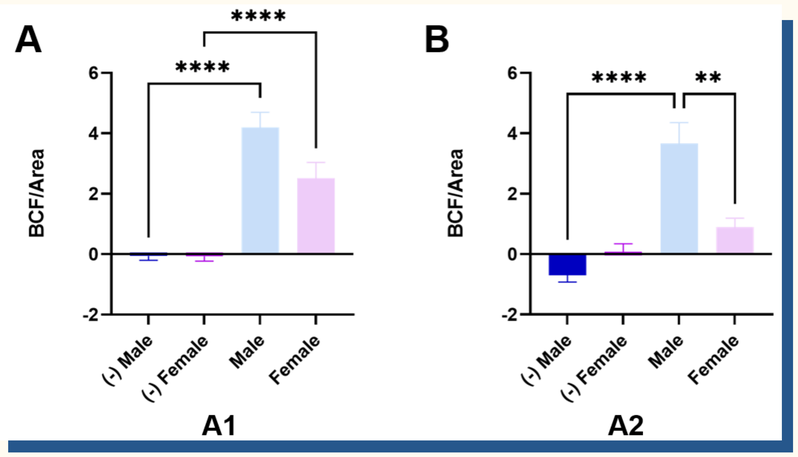


Figure 3. *Graphical representation of BCF/area values for A1 (A) and A2 (B)*

FLUORESCENCE ANALYSIS

We performed a one-way Welch's ANOVA test of our imaging data, which revealed that our average BCF/Area values were higher than their accompanied negative controls for all except A2 females. **This indicates that the CGRP expression in A1 males, A1 females, and A2 males is significant, but CGRP expression in A2 females is not.**

To test sex differences in A1 and A2, mean levels of fluorescence were analyzed using a Welch ANOVA and a Dunnet T3 multiple comparisons test for each subpopulation. Results of the ANOVA for the A1 subpopulation indicated that females have the same level of fluorescence (M = 2.51, SD = 4.87) as males (M = 4.19, SD = 4.79), p = 0.125. Results of the ANOVA for the A2 subpopulation indicate that males have a significantly higher fluorescence of CGRP (M = 3.64, SD = 5.33) as compared to females (M = 0.86, SD = 2.74), p < 0.05.

Figure 3 compared the mean BCF/area scores for both A1 and A2 across both sexes. **There was not a significant difference between males and females in the A1 subpopulation, suggesting any differences across the sexes in this location were likely due to chance. There was a significant difference between males and females in the A2 subpopulation, indicating differences in this location are not due to chance.**

POSITIVE CONTROL

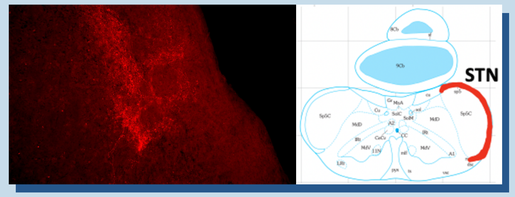


Figure 4. *Immunofluorescence of the spinal trigeminal nucleus, where CGRP is known to be highly expressed (4).*

LIMITATIONS

Sex-hormone receptor interactions with CGRP
Sex-differential CGRP expression at time of brain freezing and cryosectioning may be impacted by stage of estrous cycle in female mice, where there is a reciprocal relationship between estrogen receptor expression and CGRP expression (5).

Tissue Loss
Several samples of frozen brain tissue were lost due to mechanical error involving cryostat malfunction- portions of brain tissue were not sliced or not sliced completely.

CONCLUSION

Due to an increased prevalence of migraines amongst females and the connection between CGRP and migraines, we hypothesized that CGRP expression would be higher in NE subpopulations A1 and A2 for females. **However, with the data analyzed, we found that male mice have higher CGRP expression in A2 and there is no difference between male and female CGRP expression in A1, indicating that our data rejects the hypothesis.** This suggests that A1 and A2 are most likely not involved with CGRP in migraine pathophysiology.

FUTURE DIRECTION

Recent studies mapping the expression of CGRP throughout the brain have highlighted CGRP's mechanism of action through its three receptors: calcitonin-like receptor (CLR), receptor activity-modifying protein (RAMP1), and the receptor component protein (RCP) (4). While our results did not support our hypothesis of higher colocalization of CGRP in the female A1 and A2 noradrenergic nuclei, we should not rule out CGRP entirely but instead consider its receptor components as well. Thus, future direction for research surrounding the relationship between CGRP and the NE system may involve an investigation into CGRP's receptor components.

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