MOLECULAR SEX DIFFERENCES IN CGRP-EXPRESSING NORADRENERGIC NEURONS IN THE MOUSE A7
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BACKGROUND
Norepinephrine (NE) is a neurotransmitter that modulates many behaviors and physiological processes. NE plays a key role in both the central and peripheral nervous systems, making it a unique target of pathological treatments. NE function is affected by various genes which innervate its axons, including calcitonin gene-related peptide (CGRP).

CGRP PLAYS A KEY ROLE IN:
THE ONSET OF MIGRAINE (2)
CANCER-INDUCED ANOREXIA (3)
CARDIOVASCULAR DISEASE (4)

Prior research has confirmed the presence of six anatomically distinct NE subpopulations in the mature mouse brain (5): A1, A2, A5, A7, locus coeruleus (LC) and subcoeruleus (SubC). The NE system has also been found to have projections to some forebrain regions, including the thalamus, hypothalamus, and BNST.

HYPOTHESIS
Based on prior research regarding sex differential expression of CGRP and its receptor components, we hypothesize that female animals will show higher co-localization of CGRP and NE in A7.

METHODS
In this experiment, we used transgenic mouse brain slices (Figure 1) and double immunofluorescence labelling in order to determine the level of CGRP expression in A7 NE neurons in male vs. female animals. In the generation of these mice, Dlb::Flpo was used to recognize FRT sites in mice containing Dlb::Flpo; R::CFL::HmDi, excising the stop cassette in the NE neurons, resulting in GFP expression in all NE neurons, allowing for the visualization of all anatomical NE subpopulations under the microscope with green fluorescence.

RESULTS: DOUBLE IMMUNOFLUORESCENCE OF GFP AND CGRP IN A7 REGION

MALE

FEMALE

STATISTICAL ANALYSIS: In order to determine if the sex differential CGRP expression we found was significant, we performed an unpaired two-tailed T-test (Figure 6) using the standardized fluorescence values for the male and female A7 region. The p-value we found was 0.68 (in males = 10, in females = 10), which is much higher than the p-value required in order to declare the results of the T-test statistically significant (p<0.05). This indicates that there was no significant difference between male and female CGRP expression in A7.

In order to determine if co-localization of NE and CGRP was statistically significant, we performed a single factor ANOVA analysis (figure 6). As the p-values were > 0.05, meaning co-localization was significant in both male and female mice, with no significant sex-differential expression (Figure 6).

CONCLUSION
In this experiment, we aimed to determine if there is sex differential expression of CGRP in brain area A7 and if so, to what degree. Based on qualitative analysis, as well as statistical tests, we determined that there is significant co-expression of norepinephrine and CGRP in A7. We also found that there is no significant sex differential expression of CGRP in A7.

Our experiment did have a few limitations which could be improved upon in further research. Our sample size was only 10 male and 10 female mice. More animals could have provided us with more representative data. Additionally, it may be useful to use a positive primary antibody control in future experiments, as we did find significant co-expression in A7.

Previous research has found a link between CGRP expression and the norepinephrine system. Our experimentation supported existing data, as we did find significant co-expression in A7. This suggests that future research may focus on the expression and location of CGRP and its various receptor components in A7 as targets for therapeutic migraine-treatments, as well as for other CGRP-mediated diseases.

RESOURCES