Sex and Anatomical Differences in the Expression of CGRP across the Norepinephrine System

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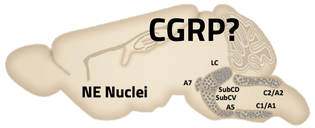
NIH NIEHS

Background

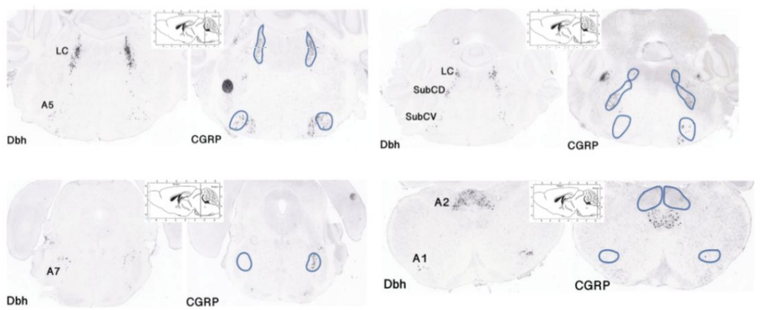
- Norepinephrine (NE) is a neurotransmitter that plays a critical role in both the peripheral (PNS) and central (CNS) nervous systems
- NE is released by the sympathetic division of the PNS during stress and chronic release can cause hypertension, cardiovascular disease, stroke, and cardiomyocyte apoptosis
  - CGRP mitigates these negative effects through vasodilation → antagonistic relationship between CGRP and NE in the PNS
- NE modulates the stress response in the CNS mostly through the locus coeruleus, where CGRP has been shown to be expressed by prior immunohistochemistry studies
  - However CGRP expression across the CNS and within the rest of the NE system has not yet been studied



- Both CGRP and NE release in the trigeminovascular system contributes to the onset of migraines via the trigeminovascular system suggesting a possible CNS pathophysiological connection
- Migraines and stress-related psychiatric disorders are both more prevalent in females, suggesting the possibility of molecular sex differences in NE and CGRP systems

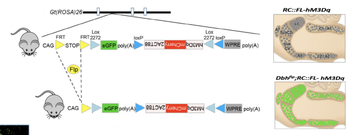
Aims and Hypotheses

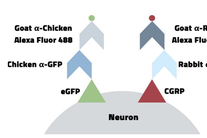
- We aim to map the expression of CGRP across the entire NE system to quantify and compare CGRP expression between NE nuclei and sexes
- We hypothesize CGRP is expressed in all NE nuclei except A1/A2 with highest expression in the LC based on observational data from the *Allen Mouse Brain Atlas* (AMBA) (shown below)
- We also hypothesize higher CGRP expression in females based on the idea that if stress induces migraine through CGRP release from NE neurons and females have higher prevalence of stress-induced migraines and stress-related psychiatric disorders, then females will exhibit higher CGRP expression across the NE system

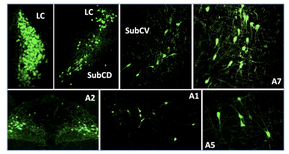


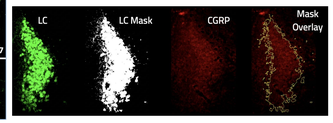
Methods

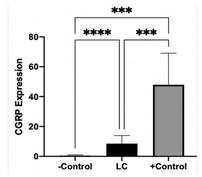
1. Transgenic mice (N=10; n=5 each sex) express eGFP in all NE neurons
2. Dual immunohistochemistry (IHC) was used to label eGFP (green) and CGRP (red)



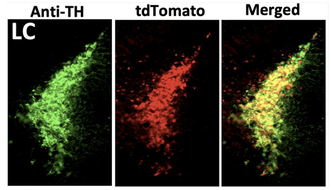








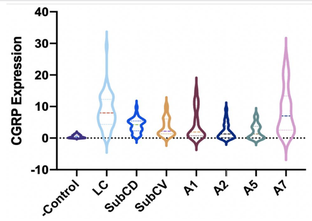
3. A mask was created for each NE nucleus and overlaid on the red anti-CGRP images from the same visual field
4. CGRP expression was quantified by measuring red signal pixel intensity per unit area of NE neurons
5. Negative and positive controls confirmed our analysis approach
6. We used an alternative transgenic approach to qualitatively confirm our imaging analysis results
   - Transgenic mice (N=2; n=1 each sex) express tdTomato in all CGRP neurons which is red
   - IHC labeled tyrosine hydroxylase (TH) green which is expressed in all NE neurons

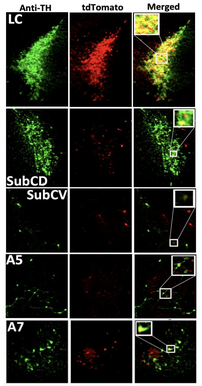


Results

Main study shows CGRP expression in ALL nuclei except A5; does not align with hypothesis
- Highest expression in LC, but not higher than A7

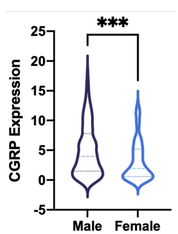
CGRP-tdTomato data qualitatively confirms highest expression in LC and expression in SubCD, A7 & SubCV
- Some CGRP-tdTomato expression in a few A5 neurons that warrants further exploration

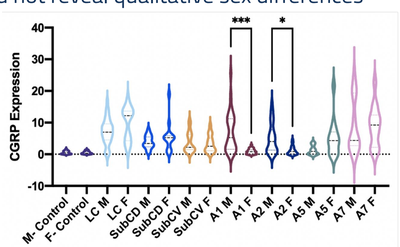




Main study found limited sex differences:
- Overall NE system: males > females; contradicts hypothesis
- ONLY A1/A2 had significant sex differences (male > female)
  - A1/A2 females not above background

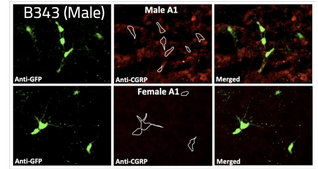
CGRP-tdTomato data did not reveal qualitative sex differences

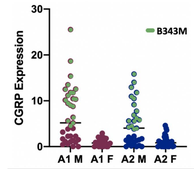


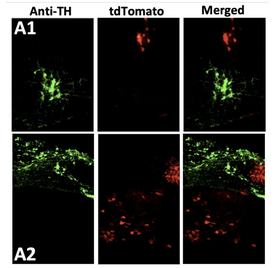


Results: A1/A2 CGRP Expression

- CGRP-tdTomato* data: no qualitative co-expression in either sex → supports hypothesis
- Main study: A1/A2 females did not show co-expression
  - ONLY A1/A2 males had significant co-expression
    - Male animal B343 = high background red signal
    - Increase N and explore outlier analyses



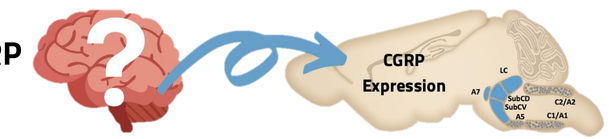




*Only male images, but females had same pattern of CGRP expression

Conclusions

- Anatomical evidence of CGRP expression in all NE nuclei except A1, A2, & A5
  - Perhaps CGRP is released from NE neurons, but still unclear HOW they interact
- Sex differences findings are contradicted by a possible lack of CGRP in A1/A2
  - To confirm no sex differences, we must increase N and perform outlier analyses
- Although we did not identify sex differences, higher prevalence of migraines and psychiatric disorders illustrates the importance of investigating molecular sex differences in these two systems for the development of sex-specific treatments



Limitations and Future Directions

Limitations:
- Qualitative CGRP-tdTomato data
- Gives anatomical evidence of co-expression, but does not answer HOW these two systems interact
- Did not control female estrous cycle

Future Directions
- Increase N and add controls to quantify CGRP-tdTomato data
- Map CGRP receptor expression and CGRP expression in NE projections
- Control for female estrous cycle

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