Sex Differences in Tyrosine Hydroxylase Expression Within the Norepinephrine System of the A1 Anatomical Region in Mice

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Background

Norepinephrine System:

- Noradrenergic neurons are a group of neurons originating in the locus coeruleus of the brainstem and are involved in the regulation of several physiological and behavioral functions.
- These neurons release the neurotransmitter norepinephrine (NE) and play a significant role in modulating various brain functions, including attention, arousal, anxiety, and stress responses.
- Dysfunctions in the noradrenergic system have been implicated in several psychiatric and neurological conditions, including Parkinson's disease and Major Depressive Disorder.

Tyrosine Hydroxylase:

- NE neurons express the enzyme tyrosine hydroxylase (TH), which is required for the synthesis of catecholamines, including dopamine, norepinephrine, and epinephrine. TH is the rate-limiting enzyme in the biosynthesis of catecholamines and is considered a biomarker of noradrenergic neurons.

A1 Anatomical Region:

- Noradrenergic neurons are primarily located in two regions of the brainstem, the locus coeruleus (LC) and the A1 region. The LC is the largest noradrenergic nucleus in the brain and is involved in the regulation of several physiological and behavioral functions, including the sleep-wake cycle, attention, and stress responses.
- The A1 region is a smaller nucleus located in the rostral ventrolateral medulla and is involved in the regulation of cardiovascular function and blood pressure.

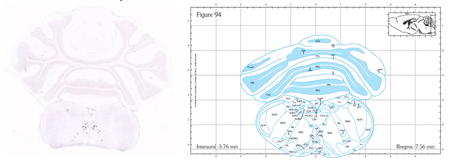

Figure 1. *Allen Mouse Brain Atlas (Dbh expression on coronal slice)* and *Franklin and Paxinos Mouse Brain Atlas* Schematic Representation of the A1 region of the Central Nervous System.

Research Goal and Hypothesis

Goal:

- To compare sex differences in tyrosine hydroxylase and norepinephrine expression in the A1 region and relate findings to neurological disorders

Hypothesis:

- We hypothesized that TH expression will be greater in female A1 regions rather than male A1 regions. This hypothesis was built upon findings from previous studies suggesting that female hormones, such as progesterone, increase A1 norepinephrine neurons' activity and TH expression.

Methods

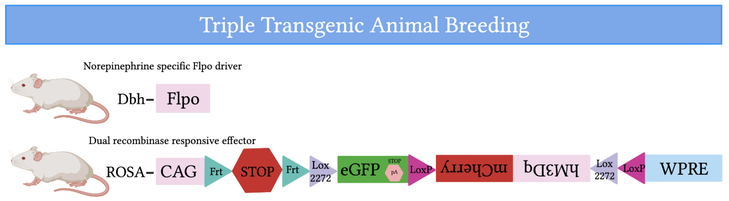

Figure 2. Schematic of triple transgenic model animals used in our project. Mice are Dbh-Flpo positive and effector positive. All NE neurons express GFP (green fluorescence).

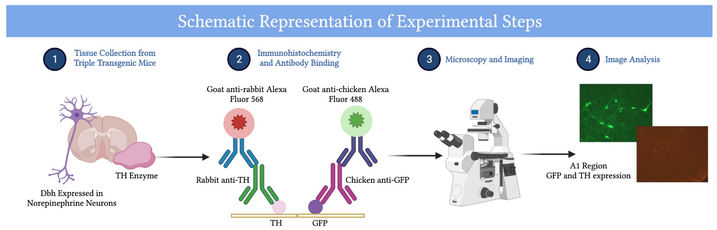

Figure 3. Experimental Steps. After first creating the triple transgenic mice models, the brain tissue was analyzed using immunofluorescence to detect GFP expression and tyrosine hydroxylase (TH) expression. Brain tissue was visualized using an epifluorescence microscope (Nikon ECLIPSE Ts2). Images were taken using NIS Elements software to visualize GFP (biomarker of NE) and TH expression.

Results

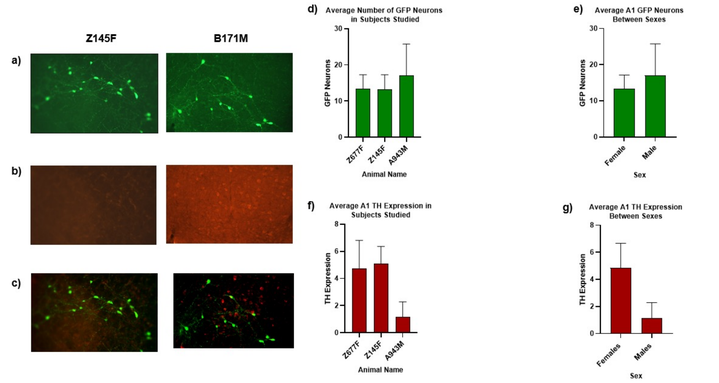

Figure 4: Comparisons of NE Neuron Counts and TH Expression between the Sexes. Images depict GFP expression (a), TH expression (b), and co-expression (c) in subjects Z145 F and B171 M. Graphs depict average NE neuron counts per 40 µm tissue slice between experimental subjects (d) and between the sexes (e), as well as average BCF/area values between experimental subjects (f), and the sexes (g).

Imaging and Statistical Analysis:

Using ImageJ software, TH expression was quantified in each 560 nm image in units of background-corrected fluorescence per area (BCF/area). NE neurons within each 470 nm image were quantified through manual counting.

D'Agostino-Pearson tests confirmed that male and female data for both TH levels and NE neuron counts possessed normal distributions. Unpaired t-tests revealed that female subjects possessed significantly higher A1 TH expression than their male counterparts; however, differences in average NE neurons per image were not statistically significant.

Project Significance

Gaps in Neuroscience Research Regarding Sex Differences:

- For a long time, research in neuroscience largely focused on male subjects with the assumption that the findings would also apply to females. However, there are fundamental sex differences in the anatomy and function of noradrenergic neurons.
  - Greater noradrenergic neurons in the LC of males compared to females
  - Greater stress response in males
- These findings suggest that the noradrenergic system may contribute to the sex differences observed in neurological disorders. By studying sex differences in neuroscience, researchers can develop a more nuanced understanding of how the brain works and how to develop more effective treatments for both men and women. This knowledge can ultimately lead to better healthcare outcomes for all patients, regardless of their sex.

Conclusions and Future Directions

- The results support our hypothesis that females exhibit greater TH expression in the A1 region compared to males.
- This has important implications because many neurological disorders, such as Parkinson's disease, impact men and women differently. For example, men are twice as likely to develop PD compared to women.
- Researching sex differences at the level of the norepinephrine system has the potential to discover why the intensity of symptoms, prognosis, time of onset, and more vary between men and women.
- Future studies should focus on additional molecular differences between men and women at the level of the central nervous system and translate the findings to create patient-centered treatment plans.
- Additionally, understanding how hormonal and neurotransmitter systems are influencing sex differences can allow medical personnel and pharmaceutical companies to make educated decisions regarding therapeutic drugs.

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