Investigation into Tyrosine Hydroxylase Expression in the Locus Coeruleus as it Relates to Neurodegenerative Disease

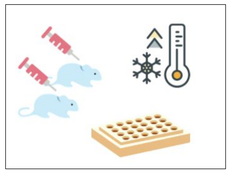
THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Gabriella M. Hesse, Ben Jones, Lily Al-Omari, Monica Sanchez

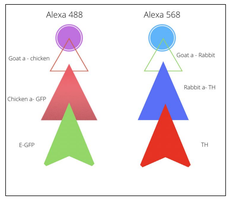
Introduction and Significance

- Of 65 year old patients, women experience a 12% risk of developing Alzheimer's Disease (AD) as compared to men who experience a 6.3% risk [Seshadri et al, 1997]. Conversely, lifetime risk of developing Parkinson's Disease (PD) in men was 2.0% and 1.3% for women [Elbaz et al, 2002]]
- Previous research has found that norepinephrine (NE) insufficiency may precede the nigrostriatal neuron death characteristic of PD [Espay et al, 2014]. In addition, the locus coeruleus (LC) is thought to be where AD originates and NE deficiency exacerbates the progression of AD [Gannon et al, 2015].
- Research has found significant differences in the expression of tyrosine hydroxylase (TH) related to estrogen that may be related to sex differences in neurological disease [Thanky et al, 2002]. Estrogen was found to downregulate TH expression in female mice but upregulate expression in male mice, indicating a sex-dependent effect of estrogen on TH expression in the LC.
- **Based on these findings, we expect to see less TH expression in the LC of female mice when compared to male mice.** Potential findings may indicate a difference in predisposition for neurodegenerative diseases, which could lead to potential avenues for treatment based on the genetic and molecular differences between male and female brains.

Methods



- Brains were perfused, frozen, and cryosectioned into 40 micron free-floating coronal sections at 120 K (-20 °C) PBS.
- Animal numbers included: 2HB D039 M, Z144 F and 2FB D039 M, Z144 F.
- Frozen slices were rinsed in 0.1 M PBS solution followed by 0.1% PBST for cell perforation. Samples were incubated in 5% NGS with 0.1% PBST for 1 hour.



- Dual primary antibody solution: chicken anti-GFP antibody in a 1:10,000 ratio and rabbit anti-TH in a 1:1,000 ratio with 1% NGS in 0.1% PBST. Samples were incubated overnight in the primary antibody solution at 4 °C.
- Solutions were incubated for two hours in a goat anti-chicken Alexa 488 and goat anti-rabbit Alexa 568 both in a 1:1,000 ratio with 1% NGS.
- Sections were mounted with 120 µL of DAPI and stored at 4℃.
- Slices were observed at 20x magnification. Images were taken with NIS Elements in brightfield, with a 800 ms exposure for Alexa 568 and 700 ms exposure for GFP/Alexa 488. Fluorescence analysis was performed using FIJI/ImageJ and Excel was used for calculations.

Results

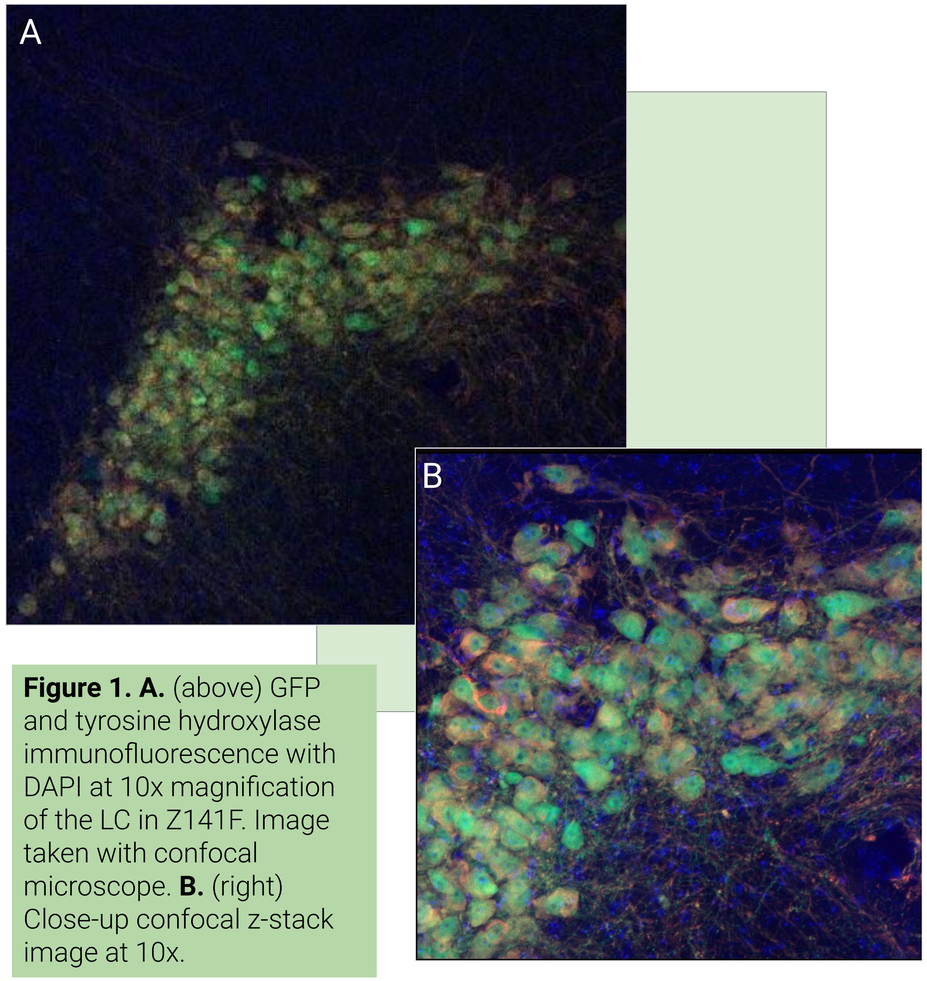


Figure 1. A. (above) GFP and tyrosine hydroxylase immunofluorescence with DAPI at 10x magnification of the LC in Z141F. Image taken with confocal microscope. **B.** (right) Close-up confocal z-stack image at 10x.

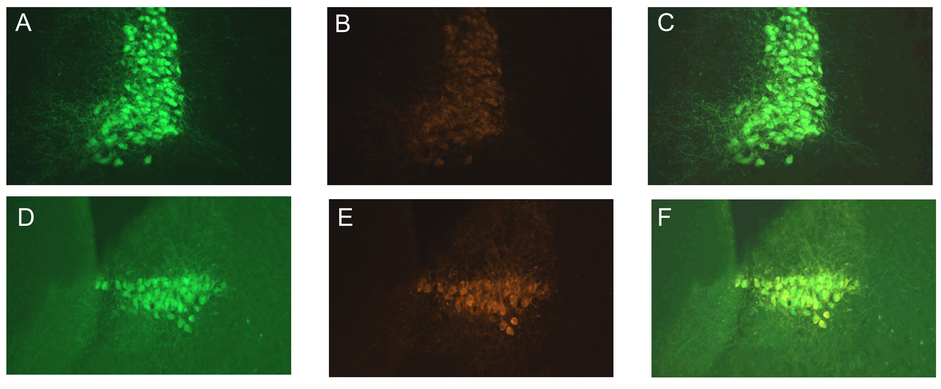


Figure 2. (below) **A.** Alexa 488 fluorescence for GFP in Z672M LC. **B.** Alexa 568 fluorescence for TH in Z672M LC. **C.** Merged fluorescence for Z672M LC. **D.** Alexa 488 fluorescence for GFP in Z141F LC. **E.** Alexa 568 fluorescence for TH in Z141F LC. **F.** Merged fluorescence for Z141F LC. Images taken at 700 ms (Alexa 488 green) and 2 s (Alexa 568 red).

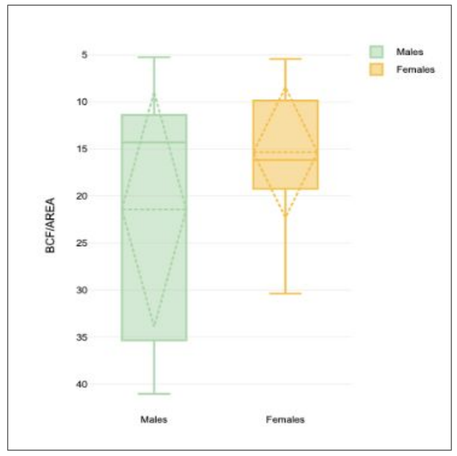


Figure 3. Boxplots of background Fluorescence (BCF) per Area for males (green) and females (orange). Females had a lower value than males but the difference was not great enough to be considered significant.

Discussion

- Due to the observed sex differences in the neurodegenerative Alzhiemer's and Parkinson's disease, we determined TH expression in the LC may be a target for continued investigation.
- We found that there was not a significant difference in the mean BCF/Area values for male (21.42) and female (15.36) samples (p value 0.1547). Values for female samples ranged from 5.43 to 30.39 with a standard deviation of 7.21, whereas male samples ranged from 5.23 to 41.05 with a standard deviation of 13.00.
- These data do not support our initial hypothesis, which predicted a smaller amount of Th expression in the male LC compared to females.

Value	Female	Male
Minimum	5.43	5.24
Q1	10.61	11.56
Median	16.18	14.31
Q3	19.04	33.85
Maximum	30.39	41.05
Mean	15.36	21.42
Standard Deviation	7.21	13.00

Table 1. (above) Data distribution of Background Corrected Fluorescence (BCF) over Area for male and female samples. Visual representation of this data is shown in *Figure 3.*