

Sex Differences in Tyrosine Hydroxylase Expression in Mouse A1 & A2 Nuclei

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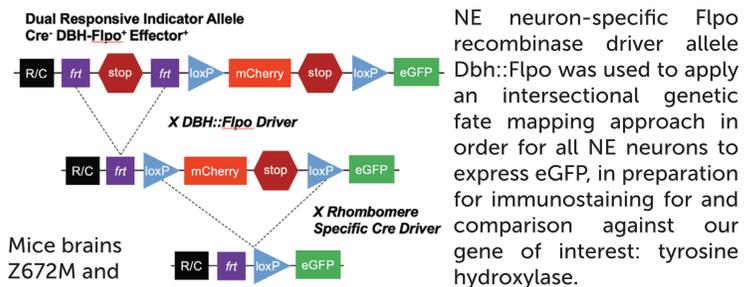
Background

In recent years, there has been a push toward analyzing sex differences in research, especially in the field of neuroscience. Most of what is known about the brain has been studied in male models and assumed to be the same in females, however, there is much research that shows otherwise. Since there are already known sex differences in the presentation and effects of the stress response, scientists decided to study the norepinephrine system to look for neuronal sex differences.¹ There has been a lot of research focusing on the locus coeruleus and many studies have found and confirmed that there are undeniable differences in the locus coeruleus of male and female rodent models. Sex differences in other subpopulations of the NE system are not as well researched. To fill this void, our team looked at the A1 and A2 nuclei of male and female mouse models, in an attempt to uncover any differences in expression of tyrosine hydroxylase, a known precursor to NE.

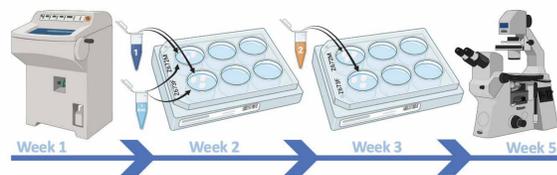
Hypothesis

Tyrosine Hydroxylase (TH) gene expression in the NE neuron subpopulations A1 and A2 will be higher in female mice compared to male mice.

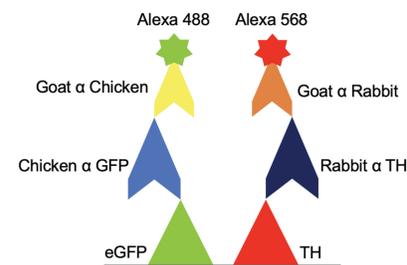
Methods



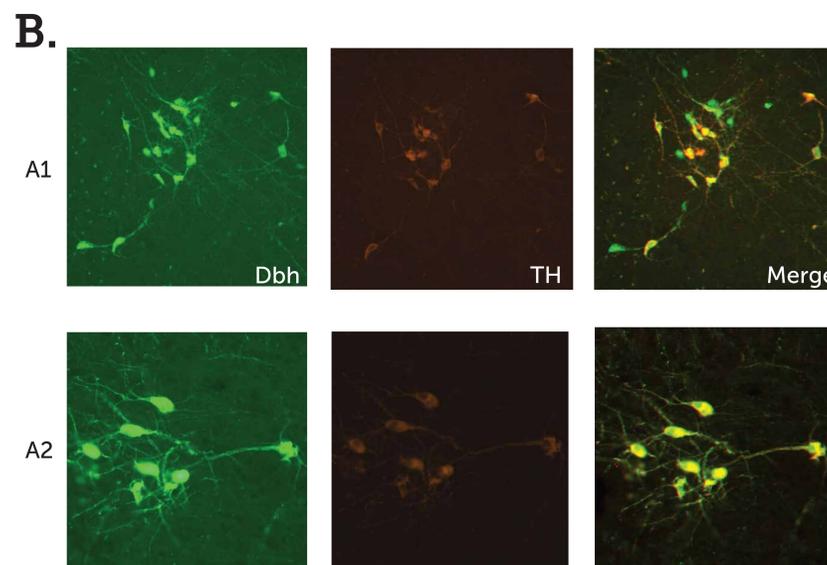
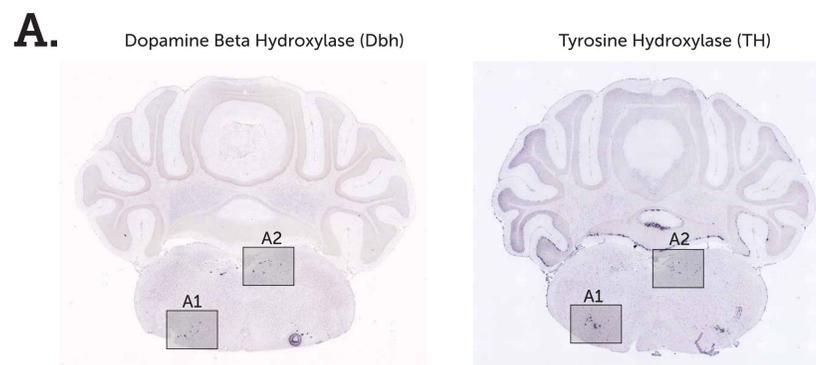
Mice brains Z672M and Z673F were cryosectioned coronally into 40 μm sections and stored in PBS in a -20 °C freezer. After a week, the primary antibody solution (with CK αGFP) and dual primary antibody solution (with Rabbit αTH and Chicken αGFP) were prepared and applied to the free-floating brain slices. One week later, the secondary antibody solution (with GtaCk Alexa 488 and GtaRb Alexa 568) was prepared and applied to the brain sections. Sections were mounted, coverslipped, and imaged using epifluorescent microscopy. Experiments were repeated with DO39M and Z144F mice brains.



Imaging TH Expression in A1 & A2

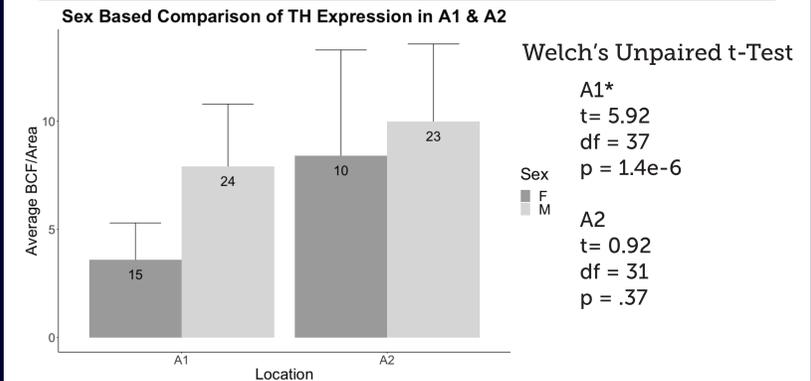


Primary antibodies Chicken α GFP and Rabbit α TH were used to bind eGFP and TH, respectively. Secondary antibodies Goat α Chicken Alexa 488 and Goat α Rabbit Alexa 568 were used to bind to their respective primary antibodies, Chicken α eGFP and Rabbit α TH, in order to further amplify signal. These signals were measured with the Nikon Eclipse Ts2 Inverted Routine Microscope.



Panel A shows the anatomical locations of the A1 and A2 nuclei in the Coronal plane. Images are from from the *Allen Mouse Brain Atlas*.² Through in situ hybridization, the expression of Dbh (left) and TH (right) were confirmed in these locations. Panel B shows immunohistochemistry staining of Dbh (left) and TH (middle) in the A1 (top) and A2 (bottom) nuclei. Merged images (right) confirmed that there was dual expression in the NE neurons of both regions.

Analysis of Sex Differences



TH expression was measured in A1 & A2 nuclei of male and female mice. Data existing outside of the interquartile range were excluded from statistical analysis. A Welch's Unpaired t-Test was conducted comparing expression between males and females in A1 & A2 separately.

Male mice had significantly more TH expression as compared to female mice in A1 nuclei ($p < 0.05$)*. Whereas there was no significant difference in TH expression between male and female mice in A2 nuclei ($p > 0.05$).

Outcomes

We found no significant difference in TH expression between sexes in the A2 region. In the A1 region, TH expression was observed to be higher in males than in females.

Acknowledgements



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References

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- Allen Institute for Brain Science (2004). *Allen Mouse Brain Atlas Dbh - RP_Baylor_102719 - coronal*. Available from mouse.brain-map.org.