Sexual dimorphism of tyrosine hydroxylase expression and noradrenergic projections to the basolateral amygdala

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Abstract

Dysregulation of the norepinephrine (NE) and dopaminergic systems have been implicated in the pathology of psychiatric disorders. Tyrosine hydroxylase (TH) is an enzyme that catalyzes the conversion of tyrosine to L-DOPA, a precursor for dopamine (DA) and NE. Although evidence has shown notable differences in the incident rates of psychiatric disorders between men and women, little remains known about the sexual dimorphisms of TH expression and noradrenergic projections to the amygdala, a forebrain structure known for modulating fear and stress[1]. In this study, we used immunohistochemistry (IHC) and fluorescence microscopy[2] to examine sex differences in the expression of TH in the NE system as well as noradrenergic projections to the amygdala. We found significant TH expression and noradrenergic projections to the amygdala, with males demonstrating greater expression relative to females. These results contradict our initial hypothesis, indicating possible misconceptions regarding the role of TH and NE expression in the amygdala and the subsequent implications this has in modulating stress responses. The findings from this study are fundamental to our understanding of sex differences in the NE system and present new avenues for research in sex-specific treatment of psychiatric disorders.

Background

Norepinephrine (NE) is a neurotransmitter that is released in response to stress and other stimuli and regulates the body's physiological responses to stress. Tyrosine hydroxylase (TH) is an enzyme that plays a crucial catalytic role in the synthesis of dopamine, which is a precursor to norepinephrine in the body [3,4]. There remain deficits in understanding both TH and NE in current research, including sex differences, that fail to specify how TH is expressed in the NE system. Although these differences may, in part, be explained by socioeconomic factors, neuroscience research has historically neglected to consider sex as a variable [5], and as such, has left significant deficits in our understanding of well-studied neuroscience topics. As new research in the field emerges, however, the issue of sex bias and omission persists[5]. Because of the known differences in male and female biology, differential incidence rates of psychiatric disorders among women and men[6], and the critical role of TH as a precursor to NE, it is important to map TH expression across both sexes. The amygdala is understood to modulate fear and anxiety responses and is known to have NE projections that contribute to aversive, anxiety-like behavior[7]. This is particularly important for understanding sex differences in psychiatric disorders and may have further implications for future therapeutic developments.

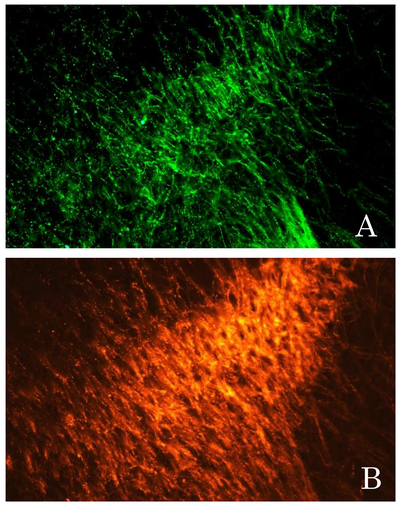


Figure 1. Expression of NE projections and TH in the amygdala in male mice. (A) eGFP-labeled noradrenergic projections, visualized at 470nm with a 1s exposure time. (B) Expression of TH at the same location, visualized at 560nm with a 5s exposure time. Images were taken at 20x objective and corrected for contrast.

Methods

Figure 2. Schematic representation of the Cre-negative, Dbh-Flpo positive dual recombinase responsive effector transgene expressed in male and female mice. The NE-specific Flpo drive recognizes FRT sites and excises the stop cassette, signaling green fluorescent protein (eGFP) in NE neurons[2,8].

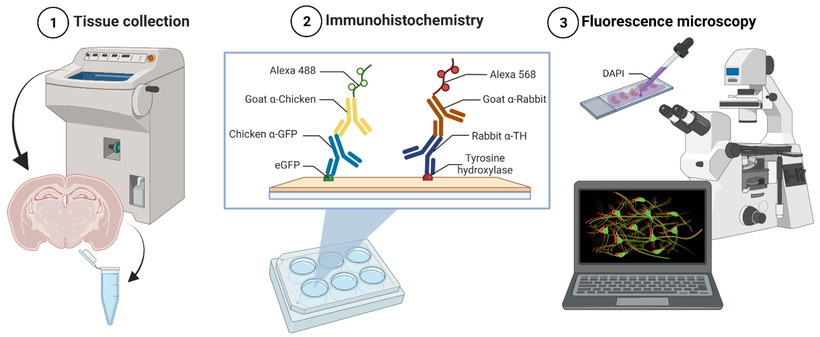
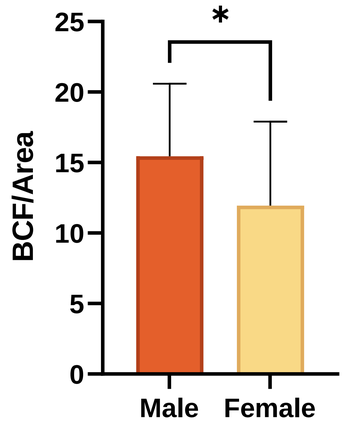


Figure 3. Experimental design[2], consisting of tissue collection, IHC, and fluorescence microscopy. Brain samples were cryoprotected and collected in 40 µM coronal slices.

Results



We determined a statistically significant (p=0.0486) difference in TH expression in the basolateral amygdala between male (N=4) and female (N=4) subjects, with males demonstrating greater levels of expression.

Figure 4. Differential expression levels of TH expression and noradrenergic projections to the basolateral amygdala in male and female subjects. Negative control experiments were performed to measure background fluorescence. Represented values (Mean ± SD) demonstrate the calculated value of background-corrected fluorescence (BCF) and were normalized by dividing this value by the area of the image to yield a measurement of average pixel intensity.

Conclusions & Future Directions

Our IHC assay was successful, as determined by comparing experimental data to that of negative controls lacking the TH-specific primary antibody and finding significant (p<0.0001) differences in expression levels between these groups. Further, we found determined significant (p=0.0486) differences in TH and NE expression in the amygdala between male and female subjects. Males generally showed higher levels of expression relative to females and no statistical difference in variance within each experimental group could be determined from our data. These findings are supported by similar studies[9], however further research is needed to understand the behavioral impact of these differences.

In the future, we would like to:

- Confirm findings by repeating the same experimental design with more subjects through multiple trials to collect more robust data
- Elucidate how differences in TH expression and noradrenergic projections are phenotypically expressed in functionally different regions of the amygdala (i.e. central, medial, lateral, etc.) and determine presence of sexual dimorphism
- Use confocal microscopy to more accurately evaluate sexual dimorphisms of noradrenergic axonal morphology in projections to the amygdala

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