Mu-opioid receptor and norepinephrine neuron colocalization in the A7 of male and female mice

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Background

- Norepinephrine (NE) plays a role in psychological disorders
- However, previous research has disproportionately been conducted with male animal models [1,2]
- These disorders are associated with stress, suggesting that sex differences in psychological disorders could arise from sex differences in stress response systems of the brain [3,4,5]
- Activation of mu-opioid receptors (MORs) on NE neurons is shown to temper Locus Coeruleus (LC) activation which could result in a decreased stress response [6,7]
- Imaging from the Allen Brain Institute shows a concentration of MOR in the A7 region of the brain
- Previous research has focused on the LC region of the brain [6]
- Our objective was to investigate potential sex differences in MOR expression in the A7 region

Hypothesis

OPRM1 gene expression colocalized with NE neurons will be greater in the A7 region of male mice than female mice

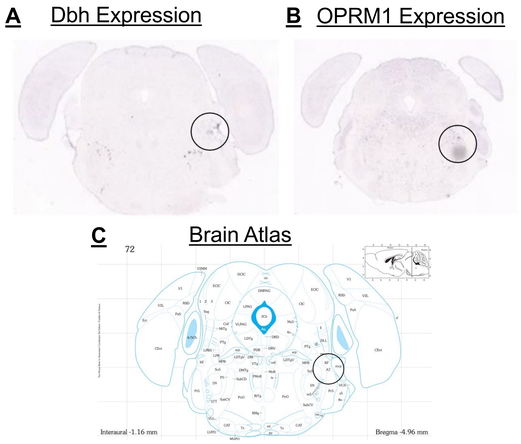

Figure 1. A Comparison of Dbh and OPRM1 Expression in A7. In-situ hybridization used for visualization of A) Dbh (NE neurons) and B) OPRM1 (μ-opioid receptors). Expression in the C) A7 is circled in black. Image Credit: Allen Institute.

Methods

Mice and Brain Slices

- Coronal hindbrain sections from 4 male and 4 female mice were analyzed
- Sections were sliced using a cryostat

Immunohistochemistry

- Slices were washed, rinsed, and incubated with antibodies to stain NE neurons and MOR
- Slices were cover-slipped with DAPI to stain all cell bodies

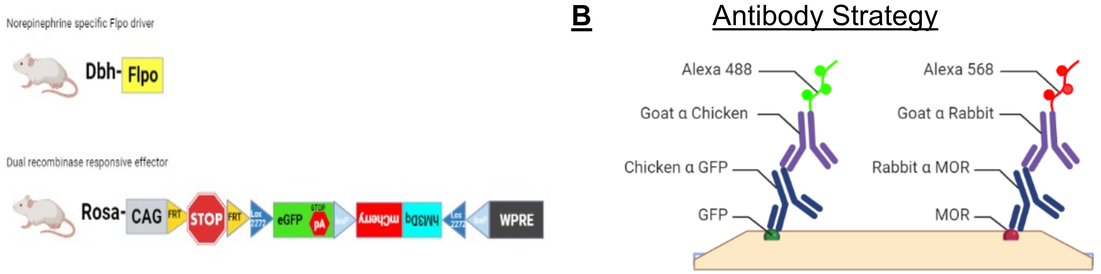

Figure 2. Flpo and Dual-antibody Strategies. A) Schematic diagram illustrating the norepinephrine specific Flpo driver (top) and the dual recombinase responsive effector (bottom). B) Schematic diagram of the dual primary antibody and secondary antibody protocol that allowed for identification of GFP and MOR expressing neurons.

Analysis & Results

A7 NE and MOR Colocalization Analysis

- A 2-way ANOVA was used to analyze the data
- There was no statistically significant difference between male and female A7 colocalization ($p = 0.501$)
- Difference between control and experimental groups was statistically significant ($p < 0.001$)

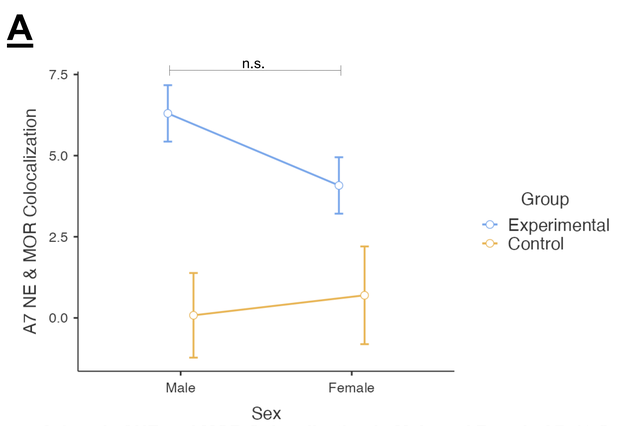

Figure 3. Level of NE and MOR Colocalization in Male and Female A7. A) Graph outlines the relationship between experimental, control, male, and female NE & MOR expression in the A7 and is derived from B) microscopy images of male and female A7 analyzed for colocalization of GFP and mCherry expression.

Image Analysis Protocol

- Slices were imaged at 20x and analyzed in ImageJ
- GFP images were used to create Region of Interest (ROI) masks that represented NE neuron populations
- Fluorescence was measured by overlaying the ROI masks on the 8-bit MOR images

Conclusions and Future Directions

- Based on evidence of increased colocalization of NE neurons and MOR in the male LC, we hypothesized a similar pattern in the A7
- We did not find statistically significant evidence to support this hypothesis. However, our data did follow the expected trend
- This could be because there is no significant sex difference in the A7, the A7 region utilizes other pathways other than MORs to regulate stress/pain response, and/or because of the small sample size
- Because the study of MOR colocalization with NE neurons in the A7 region is a novel field of study, future studies should aim to re-examine these results with a larger sample size
- Additionally, future studies should compare the magnitude of sex difference in the A7 vs. the LC and other NE cell groups
- MOR agonist binding in the A7 should also be examined for physiologic responses that differ between males and females in the intensity and integration of pain signaling as well as potential differences in behavioral responses

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