Sex Differences in Mu-Opioid Receptor Expression within Norepinephrine Neurons of the Mouse Locus Coeruleus

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Background

- Stress-related psychiatric disorders occur more often in females than males (McLean et al., 2021).
- NE neurons in the LC activate the stress response (Ross and Bockstaele, 2021).
- Mu-opioid receptors (MORs) are shown to inhibit LC activity and reduce stress (Guajardo et al., 2017).

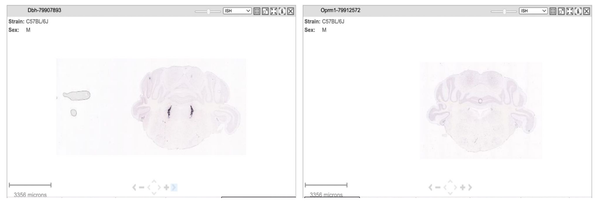

Figure 1. ***In situ hybridization showing MOR expression in the LC.*** Allen Mouse Brain Atlas in situ hybridization images showing that both Dbh (left) and MOR (right) are expressed in the LC.

- Some sex differences in MOR expression in the LC have been shown (Guajardo et al., 2017; Enman et al., 2019), but never specifically within NE neurons.
- **We expect to see greater expression of MOR within NE neurons of the male LC.**

Methods

- Mice used were transgenically modified to express GFP in all NE neurons. Mice obtained from Jensen lab.

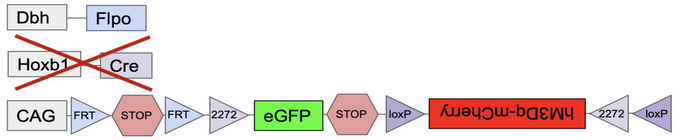

Figure 2. ***Recombinase indicator scheme.*** Driver and recombinase-responsive indicator alleles used to induce GFP expression in NE neurons. Genomes of mice used lacked the Hoxb1-Cre driver allele.

- Performed dual indirect IHC to allow fluorescence visualization of MOR and GFP-expressing NE neurons.

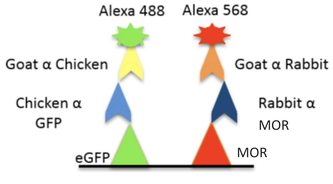

Figure 3. ***Dual indirect antibody scheme.*** Primary and secondary antibodies used to tag the target structures with fluorescent proteins.

- Imaging analysis allowed visualization and quantification of sex differences in MOR expression.

Results

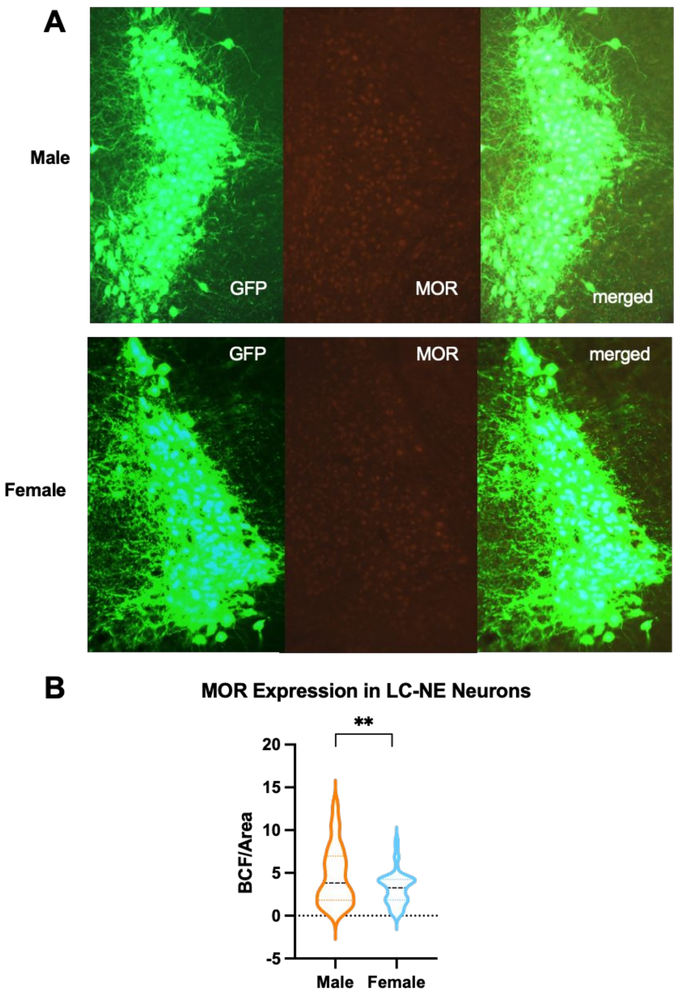

Figure 4. ***Sex differences in OPRM1 expression.*** A) Representative GFP, MOR, and merged immunofluorescence images for male (top) and female (bottom) LC-NE neurons. B) Comparative quantification of expression.

- Images show NE neurons (green) and MOR protein (red).
- MOR is expressed in LC-NE neurons of both males and females.
- Welch's t-test demonstrated significantly higher MOR expression in LC-NE neurons of males (M=4.660, SD=3.416) compared to females (M=3.201, SD=1.924), t=2.64, p=0.0100.
- These results support our hypothesis.

Control

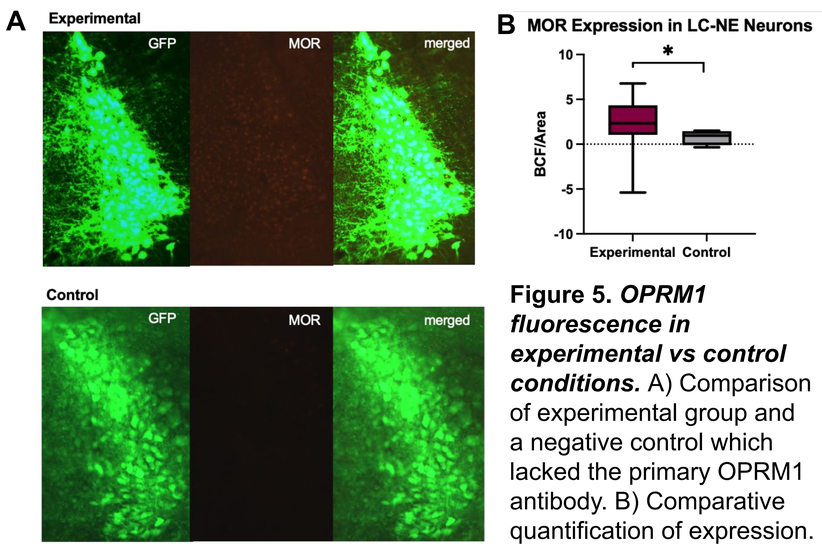

Figure 5. ***OPRM1 fluorescence in experimental vs control conditions.*** A) Comparison of experimental group and a negative control which lacked the primary OPRM1 antibody. B) Comparative quantification of expression.

- The negative control confirms the specificity of the IHC approach, contributing to confidence in the accuracy of our results.
- Mann-Whitney test revealed highly significant difference, $p<0.0001$.

Conclusions

- LC-NE neurons of male mice showed greater expression of MOR than in female mice.
- Sex differences in MOR expression within the NE system may relate to sexual dimorphism in stress-related disorders including depression and anxiety.
- Future studies could include behavioral paradigms to assess impacts of molecular findings or assess sex differences in CRFR expression in NE neurons.

Limitations

- Cannot identify MOR localization (cytoplasmic vs plasma membrane-associated).
- Photobleaching due to fluorescence microscopy.
- No evidence that sexually dimorphic MOR expression translates to cognition and behavior (post-mortem mice).

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