Sex Differences in Mu-Opioid Receptor Expression in A1 Norepinephrine Neuron Subpopulation

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Overview

- The mu-opioid receptor (MOR) plays a critical role in suppressing the stress response for norepinephrine (NE) neurons, including the A1 subpopulation.
- Sex differences in MOR expression could play a role in differences in stress regulation and therefore opiate dependence.
- Differences in MOR expression have been found based on chronic opiate exposure[1], chronic stress exposure[2], as well as other factors but only in the LC.
- Upon comparing fluorescence in male and female samples, no significant difference in MOR expression was found in the A1.

Methods

- Immunohistochemistry stain on sectioned tissue of primary and secondary antibodies and imaged using fluorescent microscopy.

Figure 1. Schematic of the recombinase-based intersectional genetic approach used in our experiment; the dual recombinase responsive effector (above) demonstrates how excision and inversion of the intervening DNA result in different color gene expression. NE-specific driver below

A

Antibody	Dilution	Manufacturer, code
Chicken Anti-Green Fluorescent Protein	1:5000	Abcam, ab13970
Rabbit Anti-Mu Opioid Receptor	1:500	Abcam, ab217766
Goat Anti-Chicken Alexa 488	1:1000	Abcam, ab150169
Goat Anti-Rabbit Alexa 568	1:1000	Abcam, ab175471

B

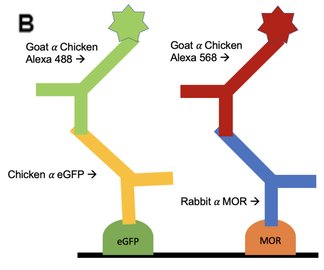


Figure 2. Table of antibodies used (A) alongside diagram representing antibody bonding during staining (B).

Results

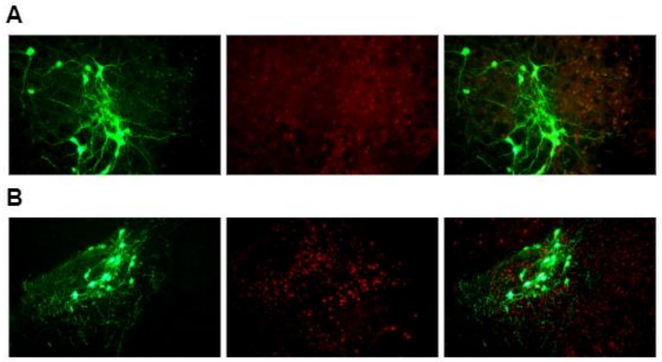


Figure 3. Colocalization of norepinephrine neurons (green) and μ-opioid receptor (MOR) (red) in the A1 region of male (a) and female (b) mice as visualized by immunohistochemistry.

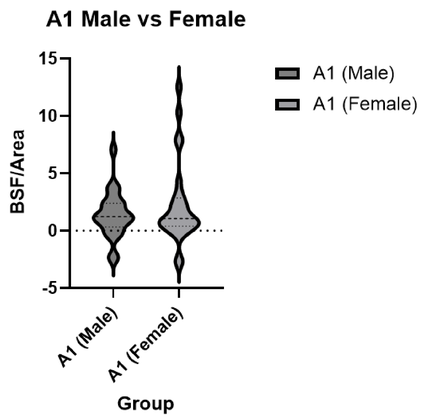
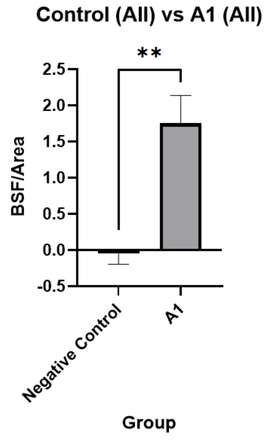


Figure 4. Comparing BSF/Area for each group, MOR is not significantly differentiall y expressed across sexes for the A1 anatomical subgroup of the NE system. Analysis complete using two-tailed t-test in GraphPad Prism[5]. Each group of mice has a different number of images analyzed due to varying numbers of NE neurons: male n=35; female n=43.

Controls

- Rabbit Anti-Mu Opioid Receptor antibody was excluded when creating primary solutions for control groups.
- Control groups showed significantly less fluorescence than experimental groups.



Conclusion

- There was no significant difference between male and female MOR-A1 expression in this study.
- While there may be sex differences in other regions within the NE system[3], the A1 subpopulation did not present a similar MOR expression dichotomy between males and females.
- Further analysis should investigate MOR-A1 expression in male and female samples to develop a greater understanding of sex-dependent differences, or lack thereof, in the A1 region.
- Testing the expression of MOR in the A1 region in live subjects exposed to chronic drug use may also provide a more integrative insight into the role of MOR in the A1 region, as it has done for MOR-LC expression[1].

Limitations

- Using dead transgenic mice samples only allowed for the testing of gene expression hypotheses, excluding potential cause-and-effect relationships of MOR in the A1 region.
- A limited number of samples were used, putting forth difficulty in drawing well-grounded conclusions from the yield of insignificant results.
- Inaccuracy during antibody solution preparation, slide mounting and imaging analysis may have had implications for the results identified.

References

1. Enman et al. *Brain Research* 2019.
2. Chaijale et al. *Neuropsychopharmacology* 2013
3. Guajardo et al. *Neuropsychopharmacology* 2017
4. Rasband, W.S., ImageJ, U. S. National Institutes of Health
5. GraphPad Prism version 8.0.0 for Windows, GraphPad Software

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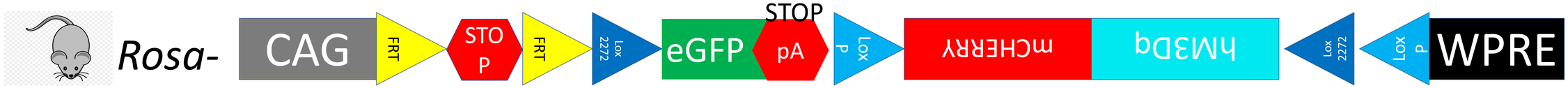
Rosa-
CAG
FRT
STO
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FRT
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eGFP
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mCHERRY
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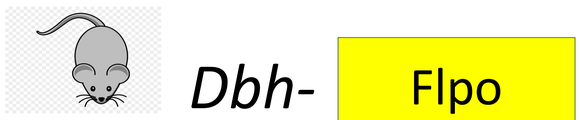
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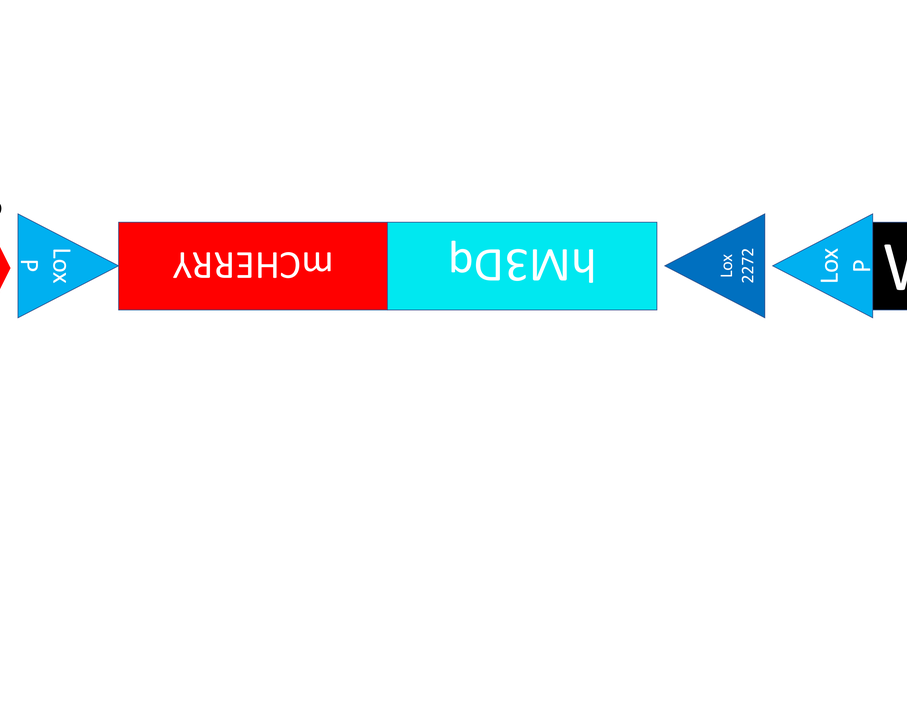
Goat α Chicken
Alexa 488 →
Goat α Chicken
Alexa 568 →
Chicken α eGFP →
Rabbit α MOR →
eGFP
MOR