Sex Differences in Mu Opioid Receptors within the A2 Region of the Norepinephrine System

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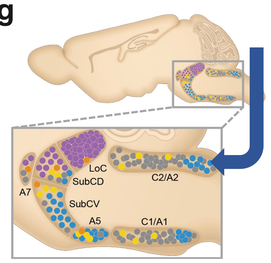
Introduction

- Norepinephrine (NE) is a chemical neurotransmitter involved in a wide range of functions such as sleep, arousal, appetite, attention, cognition, and homeostasis.[3]
- Mu-opioid receptors (MOR1) are a class of receptors involved in pain modulation and many bodily functions.[1]
- Women develop dependence to opioids more quickly than men, but men are more likely to die from opioid overdose than women.[4]
- A2, a nuclei within the NE system, has been implicated in drug dependence, addiction, and opiate withdrawal.[2]

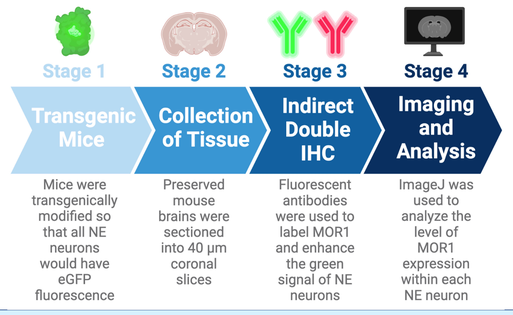
- **Our goal is to gain a better understanding of the sex differences in MOR1 expression in the A2 region to further understand addiction pathology.**



Hypothesis

We hypothesize there will be a greater prevalence of MOR1 receptors within the A2 region of male mice than female mice.

Experimental Outline



Methods

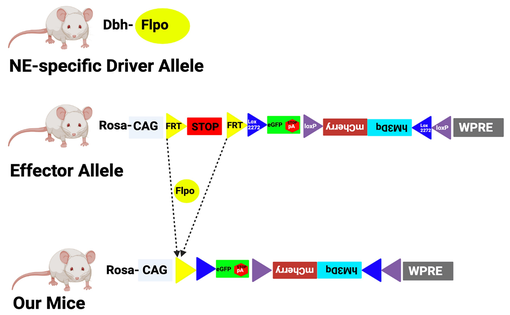


Fig 1. In order to visualize NE neurons, a NE-specific Flpo driver and a dual recombinase-responsive effector mouse line were used. Mice were Cre negative, Dbh-Flpo positive, and effector positive, which led to eGFP expression in all NE neurons.

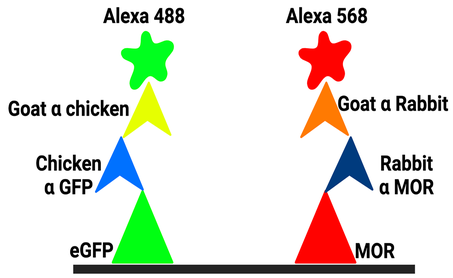
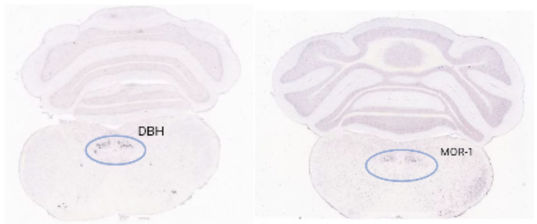


Fig 2. We utilized a double IHC strategy with this study. To enhance the green signal of eGFP we utilized Chicken Anti-GFP primary antibody (1:10,000) and Goat Anti-Chicken Alexa Fluor 488 secondary antibody (1:1000). To visualize MOR1, we used Rabbit Anti-MOR primary antibody (1:500) and Goat Anti-Rabbit Alexa Fluor 568 secondary antibody (1:1000).

Results

Confirmation of Methods:



A. A2 region with DBH (NE) expression

B. A2 region with MOR1 expression

Fig 3. Using the *Franklin and Paxinos Mouse Brain Atlas*, the A2 region of the NE system was identified, showing MOR and DBH (NE) expression.[5]

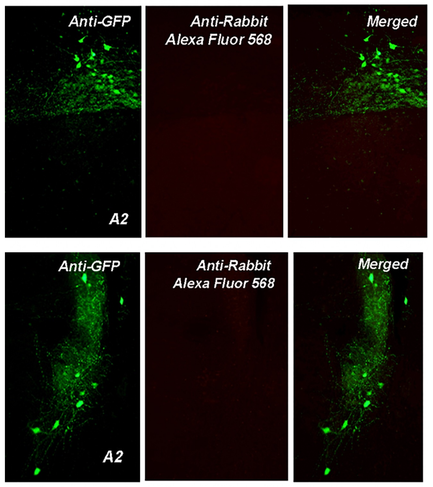


Fig 4. As a negative control, no Rabbit Anti-MOR primary antibody was used, but Goat Anti-Rabbit Alexa Fluor 568 secondary antibody was used for both sexes. There was minimal fluorescence when no primary antibody was used, showing that our protocol is effective and our secondary antibody is binding specifically.

Sex Differences in MOR1 Expression:

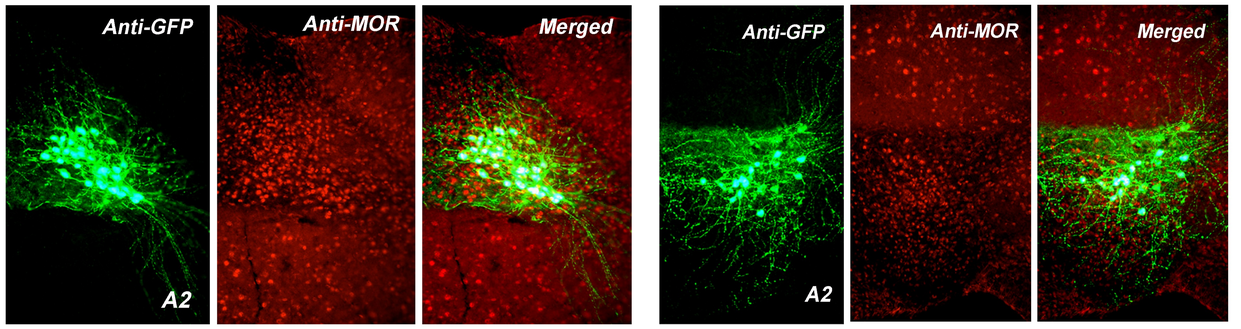


Fig 5. Comparison of MOR1 expression within A2 NE Neurons in Male and Female Mice.

Quantification of MOR1

Fig 6. MOR1 expression in Males and Females compared to Negative Controls. As determined using a Welch's One Way ANOVA and Dunnet Test, groups are significantly different from each other. Females (n =5, images analyzed = 80) have significantly greater MOR1 expression than control females (n = 1, images analyzed = 8, p=0.0368), and males (n = 5, images analyzed = 70) have significantly greater expression than control males (n = 1, images analyzed = 28, p=1.8*10^-9).

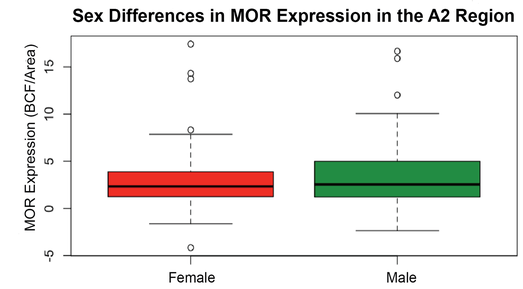


Fig 7. To identify whether there were significant differences between male and female MOR1 expression, a Welch Two Sample T-Test was used. It was found that there were no significant differences (t = -1.764, df = 117.37, p = 0.0799).

Conclusion

- MOR1 is expressed within A2 NE neurons
- MOR1 expression is not significantly different between the sexes

Future Directions

- Replicate experiment but exert more control over the animal's social environment
- Utilize other methods such as electrophysiology and MOR1 agonist and antagonist injections

References & Acknowledgements

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