

# Sexual Dimorphism of $\mu$ -Opioid Receptor Expression in Mouse A2 Neurons



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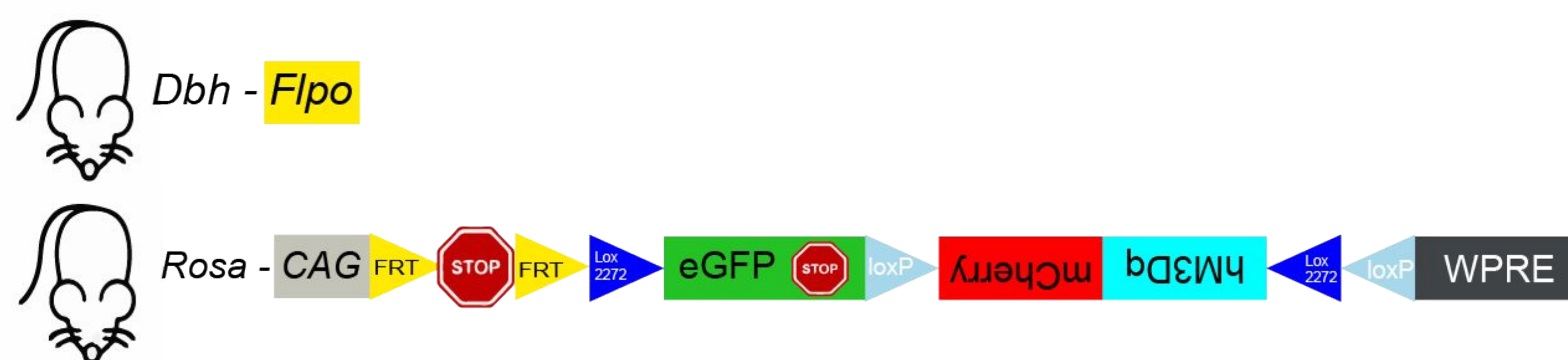
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## Overview

- Minimal research in neuroscience has investigated sex differences, despite prior research indicating that females are more vulnerable to stress-related diseases compared to males.<sup>1</sup>
- Previous studies have indicated decreased  $\mu$ -opioid receptor (MOR) function in the female rat locus coeruleus (LC), although there is a lack of research in other regions and in mice.<sup>2,5</sup>
- Furthermore, it has been implied that activation of the A2 region causes MOR-mediated norepinephrine (NE) release in the brain.<sup>3,4</sup> Therefore, our study will further prior research and examine sexual dimorphisms in MOR expression within NE neurons of mouse A2.
- **Hypothesis:** Female mice will show reduced MOR expression in NE neurons of the A2 region compared to male mice.

## Methods

### Immunohistochemistry (IHC)



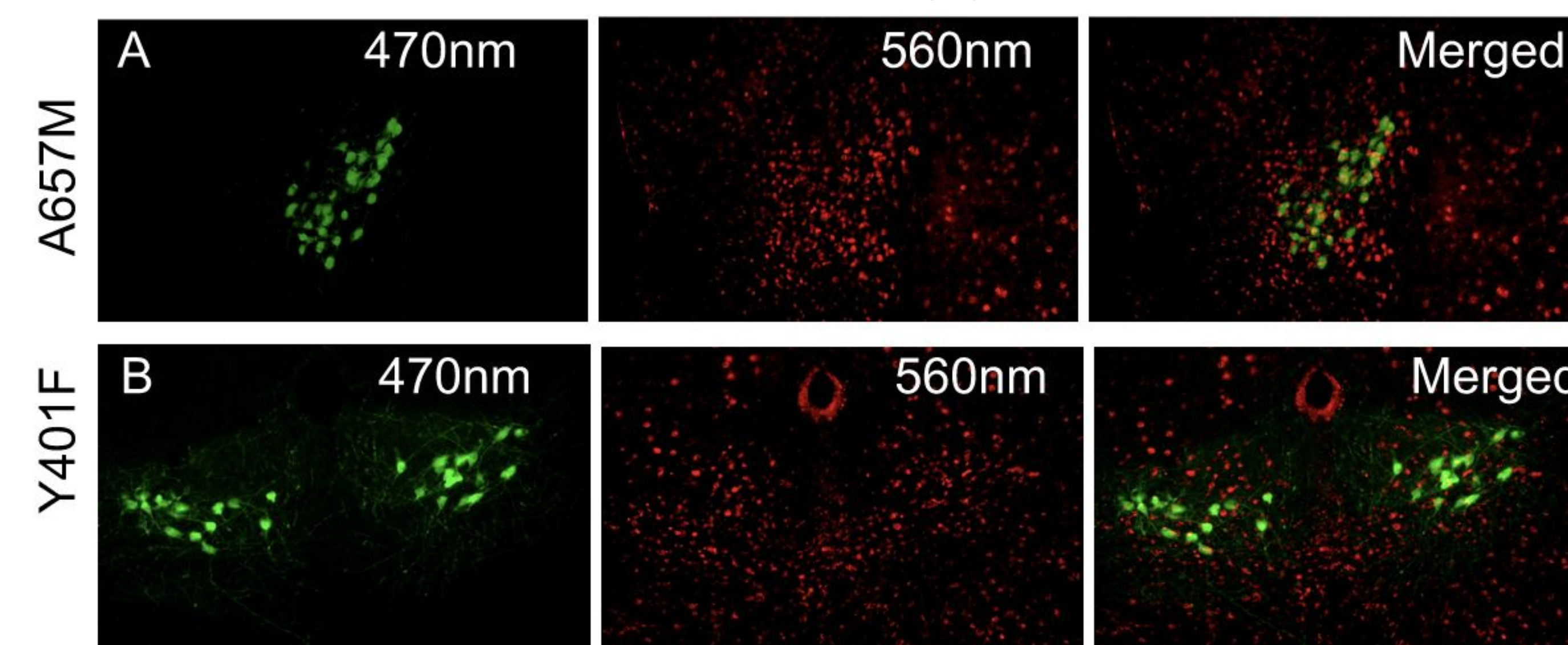
**Figure 1: Schematic diagram the transgenic mice that express enhanced green fluorescent protein (eGFP) in every NE neuron.**



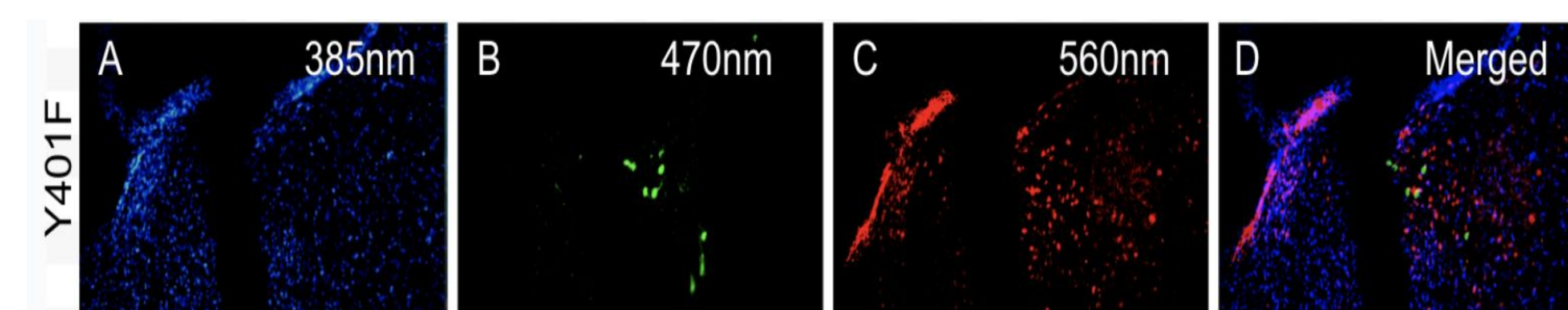
**Figure 2: Dual primary and secondary antibodies used to tag MOR and eGFP.**

## Results

### Microscopy



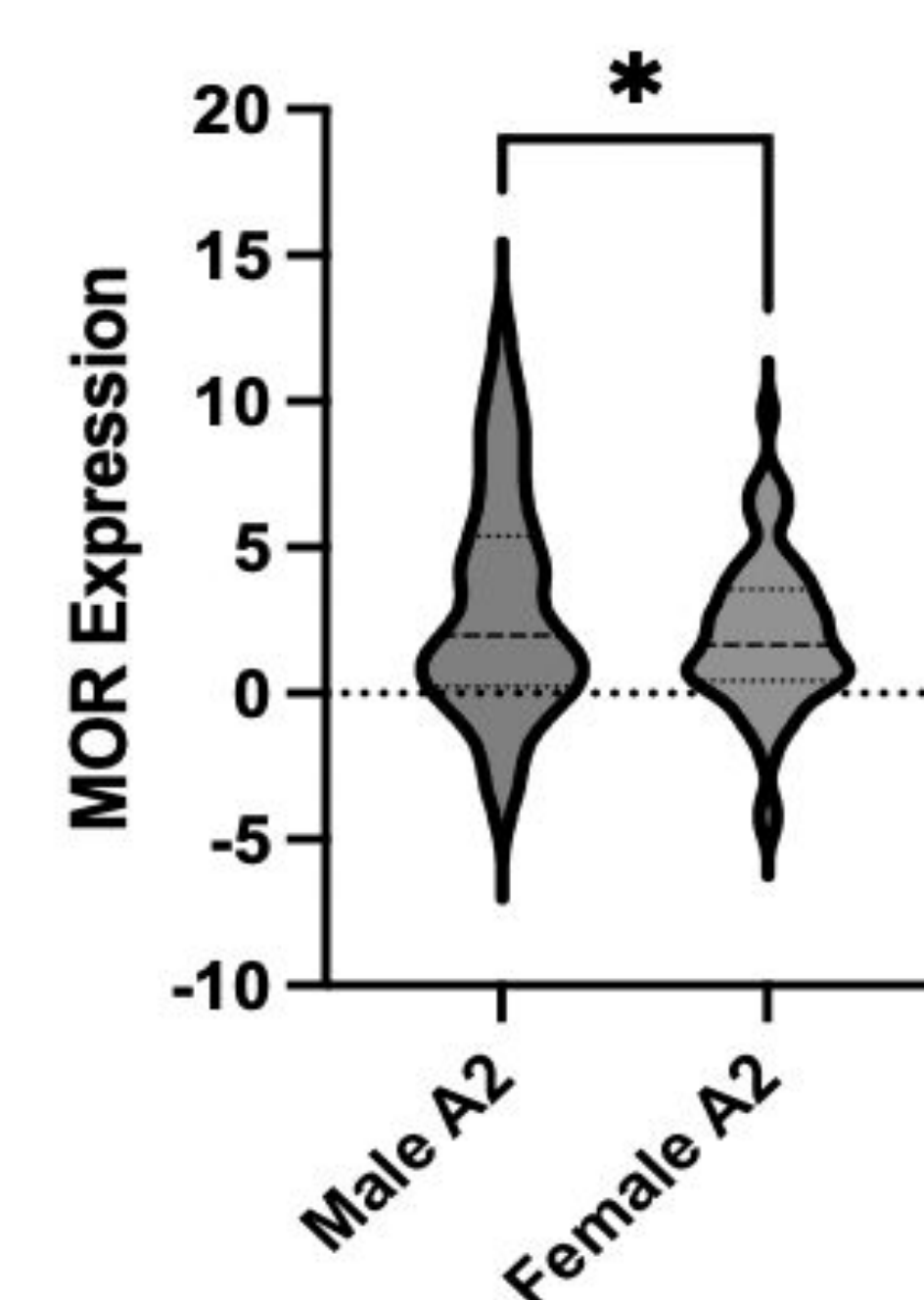
**Figure 3: Fluorescence microscopy was used to obtain images of eGFP (green) & MOR (red) in the A2 region of mice A657M (male) and Y401F (female).**



**Figure 4: Confocal images of nuclei (blue), GFP (green), and MOR (red) as well as a merged image for mouse Y401F in the A2 regions.**

Images taken show antibody stains of eGFP (green), MOR (red), and nuclei (blue). The presence of stained MOR and eGFP-expressing NE neurons (Figures 3 & 4) show that our IHC protocols were successful.

### Statistical Analysis

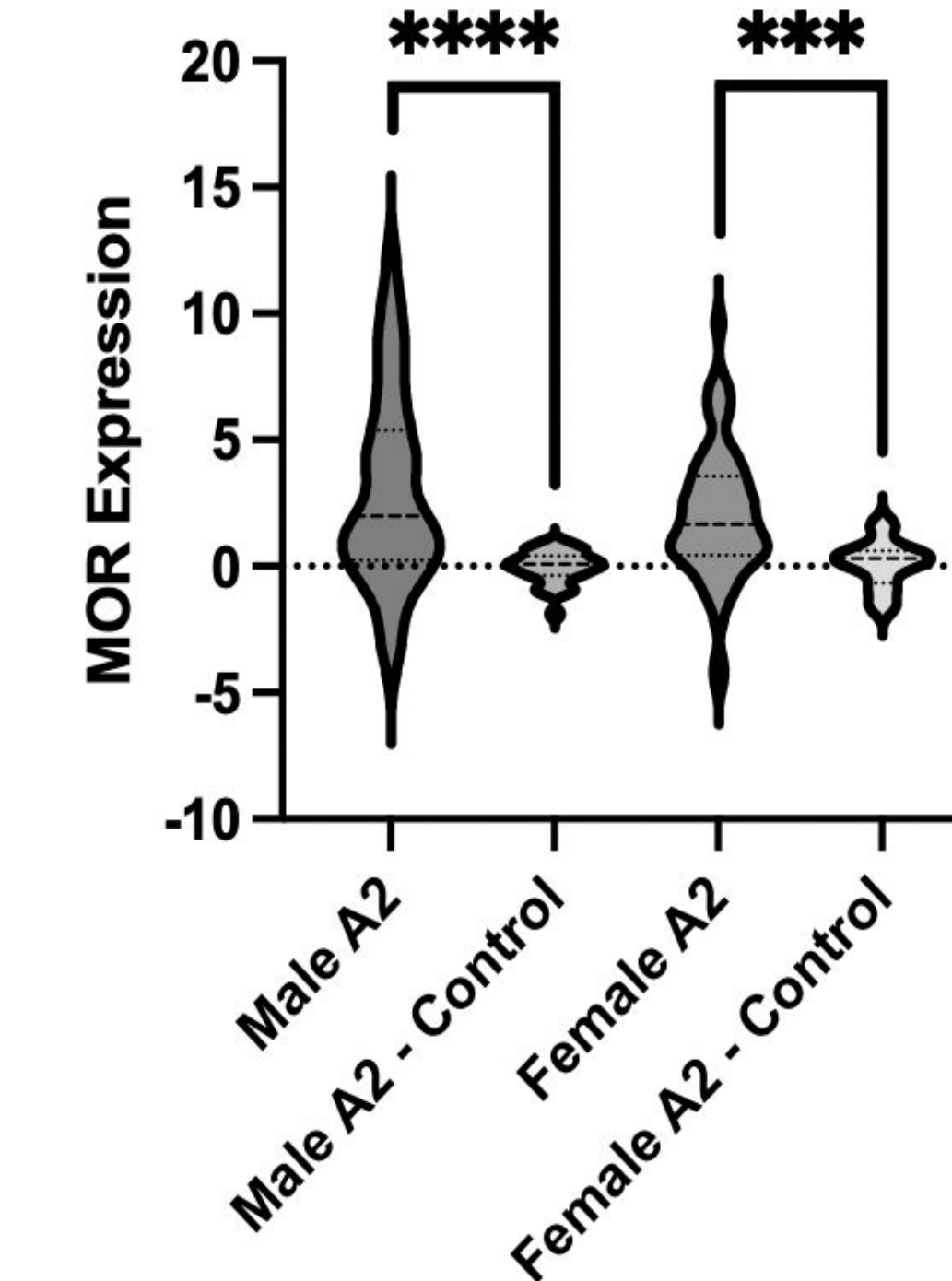


**Figure 5: Relative MOR expression between male and female mice in A2 NE neurons.**

An unpaired Welch's t-test revealed that there was a statistically significant difference in MOR expression in the A2 between male ( $M = 2.95$ ,  $SD = 3.85$ ) and female ( $M = 2.01$ ,  $SD = 2.61$ ) mice,  $t = 2.08$ ,  $p = 0.039$ . This affirms our hypothesis that there would be reduced MOR expression in female mice. This may play a role in the difference of stress regulation between the sexes in mice.

## Controls

### Sex Differences Control Data



**Figure 6: Controls used to verify image analysis and sex differences in MOR expression.**

- It is evident from the negative control data that staining of MOR was successful.
- There were significant differences between the male and female A2 MOR expressions and their respective negative controls (Figure 6).
- Unpaired Welch's t-tests were performed to confirm statistical significance (Figure 6).

## Discussion

### Implications and Future Directions

- Sex differences in MOR expression in mice A2 NE neurons were revealed by our research study, with female mice showing reduced MOR expression than male mice.
- These differences in MOR expression could contribute to differences in MOR-mediated responses in A2 NE neurons. Future studies could aim to uncover if sex differences in MOR expression in A2 NE neurons contribute to differential stress responses in males and females and stress-related disease morbidity.
- Future studies could be conducted to show if these findings can be generalized to humans.

### Limitations

- Shortened time period for our second IHC experiment, leading to a smaller sample size.

## Acknowledgements

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References