# Sex Differences in µ-Opioid Receptor-1 Expression in the Ventral Subcoeruleus of the **Mouse Norepinephrine System**

Alysa Englehardt, Andrea Myer, Rand Parrish, Zahir Sharifi, Sabrina Robertson, PhD (Advisor)

### Overview

- $\Box$  Within the NE system,  $\mu$ -opioid receptors (MOR1) play a role in modulating stress response by inhibiting LC activity (Matthes, H. W. et al., 1996; LaBuda, C. J., 2000).
- □ Historically, neuropsychiatric disorders are more prevalent in females than in males, calling for research on sex differences McLean et al. (2011)
- □ Immunohistochemistry was conducted with primary and secondary fluorescent antibody staining of coronal male and female transgenic brain sections.

Hypothesis: The Ventral Subcoeruleus (SubCV) is expected to exhibit a higher degree of MOR1 expression in female mice.

### Methods

□ Mice brains were engineered to express green fluorescent protein (eGFP) in all NE neurons.

Norepinephrine Specific Flpo Driver Dual Recombinase Responsive Effector





ROSA - CAG FRT STOP FRT 1222 EGFP EN INP hurun DAEWU LOX WPRE

Figure 1: Schematic of Cre-negative, Dbh-Flpo driver mice (Robertson, S. et al., 2013).

□ IHC methods used with primary and secondary antibodies to visualize NE expression via eGFP and MOR1 expression via mCherry fluorescence.



Figure 2: Schematic of primary and secondary antibodies used for eGFP via Alexa 488 and our GOI (MOR1) via Alexa 568 (Robertson, S. et al., 2013).

Table 1.	Primary	and	Secondary	ľ
----------	---------	-----	-----------	---

	Antibody	Host Species	Dilution	Manufacturer, Catalog #
Primary Antibodies	Chicken anti-GFP	Chicken	1:10,000	Abcam, ab13970
	Rabbit anti-MOR	Rabbit	1:1000	Abcam, ab217766
Secondary Antibodies	Goat anti- chicken alexa 488	Goat	1:1000	Abcam, ab150169
	Goat anti- rabbit alexa 568	Goat	1:1000	Abcam, ab175471

# Results



B





**Panel A.** Dopamine beta hydroxylase (Dbh) expression (left), MOR1 expression (middle), and overlay of Dbh and MOR1 expression (right) in SubCV region of Y170 female transgenic mouse.



Panel B. Control: Dbh expression (left), MOR1 expression (middle),





overlay of Dbh and MOR1 expression (right) in SubCV region of Z726 Male transgenic mouse.









Panel D. Control: Dbh expression (left), MOR1 expression (middle), and overlay of Dbh and MOR1 expression (right) in SubCV region of B345 Male transgenic mouse.



We would like to thank Dr. Sabrina Robertson for her dedication to her students' success throughout this project as well as the NIEHS for teaming with NSCI 278 to conduct this research.





and overlay of Dbh and MOR1 expression (right) in SubCV region of B346 female transgenic mouse.





**Panel C**. Dbh expression (left), MOR1 expression (middle), and





females.

Limited sample size and statistical power of male and female sections In vitro approach limits total observation of neuronal interactions that express both NE and MOR1 receptors



THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

# Analysis



Figure 3. ANOVA statistical analysis indicates a significant elevation in both female and male MOR1 expression in SubCV NE nuclei compared to their negative controls. Statistical significance is not established between male and female experimental subjects.

# Conclusion

Difference in MOR1 expression in SubCV NE nuclei was not significant between males and

□Both male and female transgenic mice display significant MOR1 expression in NE nuclei when compared to their negative controls, indicating MOR1 presence in NE neurons.

### Limitations