Sex-differential expression of mu-opioid receptor 1 in noradrenergic neurons of the A7 and paraventricular nucleus of the thalamus

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INTRODUCTION

- Norepinephrine (NE) neurons in the A7 brain region contribute to upper airway patency during sleep[1]
- A7 NE neurons project to the paraventricular nucleus of the thalamus (PVT)[2], a brain region which regulates sleep-wake cycles[3]
- Males and females have different rates of sleep disorders with chronic opioid use[4], which suggests that sex-differential opioid-mediated modulation of NE neurons may affect sleep-wake regulation
- The NE system has been shown to have sex-differential expression[2]
- Little research has been done investigating sex differences in mu-opioid receptor 1 (MOR1) expression in the A7 and PVT

RESEARCH QUESTION

Do males and females have differential expression of MOR1 in NE neurons of the A7 and PVT?

METHODS

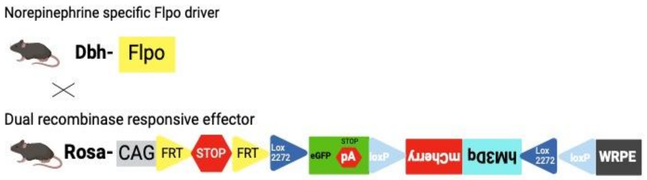
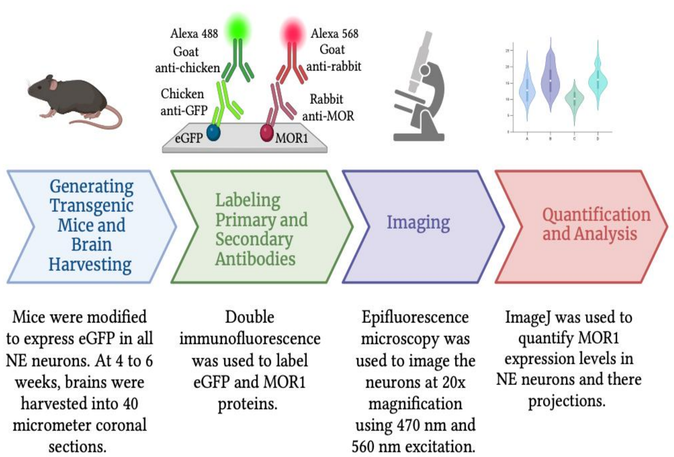


Figure 1. Transgenic mouse scheme. Transgenic mice were generated by breeding mice with the Dbh-Flpo allele with mice containing the RC::FL-hM3Dq allele, leading to eGFP expression in norepinephrine neurons.



RESULTS

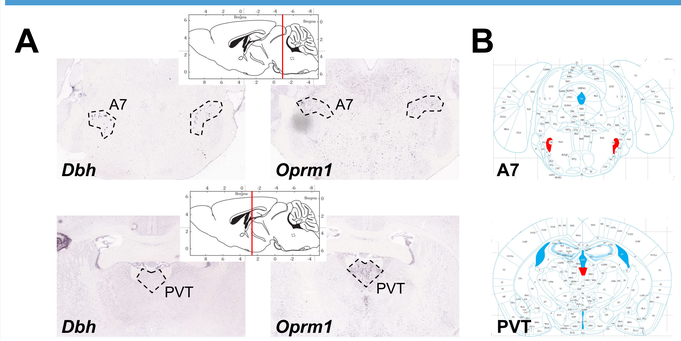


Figure 2. Preliminary data from the *Allen Mouse Brain Atlas* shows *Dbh* and *Oprm1* mRNA expression is present in the A7 and the PVT. (**A**) *In situ* hybridization stains from the *Allen Mouse Brain Atlas* of *Dbh*- or *Oprm1*-expressing neurons (dark purple) in C57BL/6J male mouse coronal sections using an antisense RNA probe. Sagittal plane illustrations from the *Frank and Paxinos Mouse Brain Atlas*. (**B**) Illustrations from the *Frank and Paxinos Mouse Brain Atlas* showing anatomical locations of the A7 (red, top) and PVT (red, bottom) in the coronal plane.

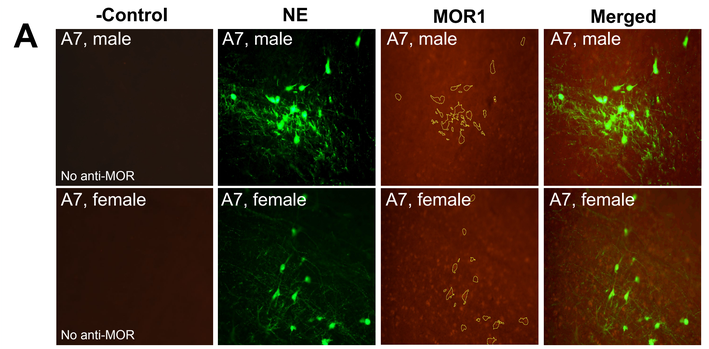


Figure 3. Males have higher MOR1 expression in A7 NE neurons than females. (**A**) IHC staining of eGFP (green) and MOR1 (red) in the A7 of male and female mice. (**B**) Quantification of MOR1 fluorescence intensity in A7 NE neurons of male control (n=2, images=4), female control (n=1, images=3), male (n=9, images=23), and female (n=5, images=12) mice. Data analyzed using Welch's ANOVA followed by Dunnett's T3 test (*P<0.05, ***P=0.0001, ****P<0.0001).

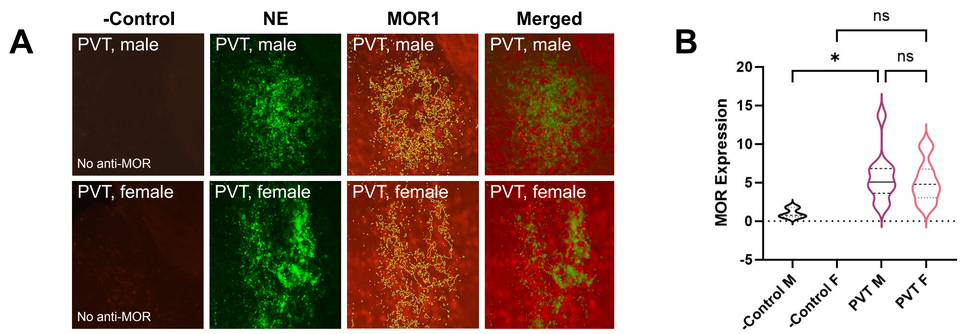


Figure 4. Males and females have no difference in MOR1 expression in PVT NE neurons. (**A**) IHC staining of eGFP (green) and MOR1 (red) in the PVT of male and female mice. (**B**) Quantification of MOR1 fluorescence intensity in PVT NE neurons of male control (n=1, images=3), female control (n=1, images=1), male (n=2, images=15), and female (n=4, images=17) mice. Data analyzed using Kruskal-Wallis test followed by Dunn's test (*P<0.05).

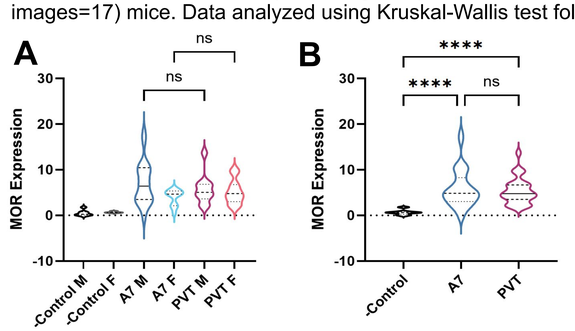


Figure 5. The A7 and PVT have no difference in MOR1 expression. (**A**) Quantification of MOR1 fluorescence intensity in A7 and PVT NE neurons of male and female mice; separated by sex, n and number of images analyzed listed in previous figures. (**B**) Quantification of MOR1 fluorescence intensity in A7 and PVT -Controls (n=5, images=15) and A7 (n=14, images=35) and PVT (n=6, images=32) NE neurons of male and female mice; sexes combined. Data analyzed by Kruskal-Wallis test followed by Dunn's test (****P<0.0001).

CONCLUSIONS

- Findings align with previous research regarding the presence of MOR1 in the A7
- Higher MOR1 expression was observed in the A7 of males compared to females as expected
- There was no significant difference between MOR1 expression in the PVT of males and females
- Sex differences in the PVT may not play a causal role in differential presence of sleep disorders by sex
- No difference was observed in MOR1 expression between the A7 and the PVT within individuals, suggesting that much of the variation in MOR1 expression occurs in the dendrites or cell bodies

FUTURE DIRECTIONS

- Experimentally confirm that the NE projections we observed in the PVT arose from the A7
- Replicate this study with a larger sample size, different NE subregions, and with more detailed methods of imaging to allow for a better understanding of specific localization of MOR1 receptors on NE neurons and their projections
- Chemogenic approaches could be used to manipulate the A7 and PVT to further study the role NE plays in modulating sleep disturbances in opioid users

LIMITATIONS

- Short research period resulted in limited samples of the PVT and A7
- Photobleaching of immunofluorescent samples may have resulted in inaccurate quantification data
- Limited data may have resulted in overgeneralization of findings

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