Sex differences in MOR expression in the A1 and BNST: Its role in sex differences in schizophrenia

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Abtract

- Norepinephrine (NE) vital in cognitive functions, including working memory, learning and memory, and memory consolidation.
- Dysfunctional μ-Opioid Receptor (MOR) contributes to negative symptoms in schizophrenia.
- Noradrenergic neurons show differences in expression between sexes.
- Immunohistochemistry and Microscopy
- Findings can be used to develop treatments to more effectively treat diseases with substantial sex-related differences.

Hypothesis

- μ-Opioid Receptor expression in the NE neuron subgroup A1 and BNST will be less prominent among male than female mice.

Methods

Mice brain tissues genetically engineered to have NE neruons express enhanced green fluorescent protein (eGFP).

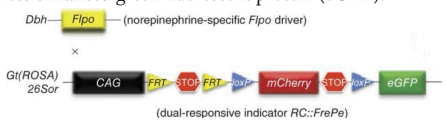

Figure 1. Schematic diagram illustrating the NE specific Flpo driver for Dbh(top) and the dual recombinant responsive effector (bottom).

Immunohistochemistry was utilized, employing primary and secondary antibodies to visualize eGFP and MOR expressing neurons during imaging.

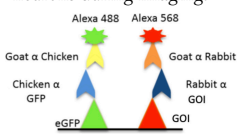

Figure 2. Dual primary antibody and secondary antibody protical allow for indentification of eGFP- and MOR - expressing neurons

NE and MOR expression were imaged at 470 nm and 560 nm respectively with a Nikon ECLIPSE Ts2 inverted microscope. MOR expression was quantified by calculating the mean pixel intensiy multiplied by the area relative to the background using FIJI software.

Background

- Dysregulated MOR signaling in noradrenergic neurons could disrupt the balance of noradrenaline, leading to alterations in cognitive function, emotional processing, and stress responsiveness, all of which are core features of schizophrenia. (Buckley et. al, 2008)
- Opioid dysregulation was first revealed to be involved in schizophrenia in the 1980s. (Ashock et. al, 2019)
- Males are at higher risk of negative symptoms compare to females with schizophrenia. Li et. al, 2022)
- In recent years there have been an increasing amount of studies with female participants showing variation between male and female patients. (Urien et. al, 2021)
- Variance in MOR could provide evidence that MOR is a causal factor in negative symtoms in men.

Results

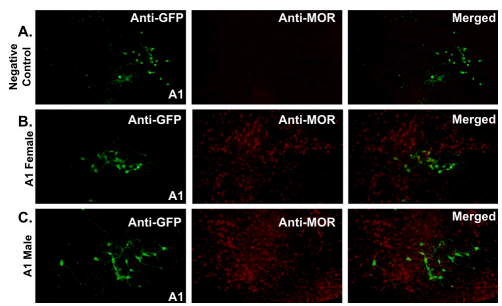

Figure 3. Quantification of MOR expression in A1 NE nuclei

A) displays the negative controls for each norepinephrine (NE) region of interest through immunohistochemical (IHC) staining, excluding the anti-MOR primary antibody.
B) and C) representative images depict successful eGFP and μ-opioid receptor (MOR) fluorescent staining through IHC in female and male mouse BNST, respectively.

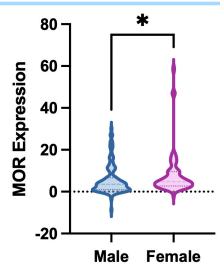

Figure 4. MOR Expression across seven NE sub-groups

This graph shows the quantification of all MOR expression data across seven NE subgroups (Locus Coereulus, SubCV, A1, A2, A7, BNST, and PVT). Unpaired T-test revealed a significant increase in female MOR expression compared to male mice (p<0.0369). Male mice N = 113; Female mice N = 107.

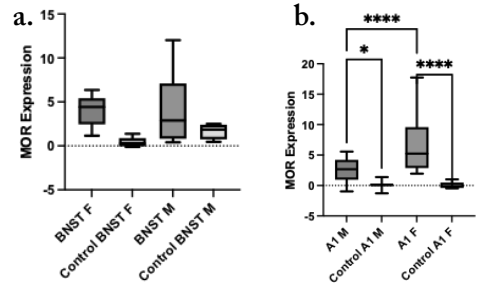

Figure 5. Graphical analysis of the difference between BNST and A1 expression in male and female mice.

a) This figure displays the difference between male and female expression of MOR in the norepinephrine projections in the bed nucleus of the striatial terminalis. There were no significant data found in these findings (p=0.999).
b) This figure displays the variation between male and female expression of MOR in the norepinephrine sub-group A1. There was significant data found between the sexes (p<0.0001)

Conclusion/Discussion

- There is a significant difference in MOR expression between male and female mice in the A1 subregion with male mice showing lower levels of expression
- There was no significant difference in MOR expression between male and female mice in the BNST
- Our hypothesis was partially supported and our findings can ultimately impact the way that schizophrenia is treated between men and women
- We can produce treatment that is specifically tailored around increasing MOR expression/and or amplify the receptors already present in men that could potentially curb the negative symptoms that men are more susceptible to
- Future directions include doing the same work on schizophrenic mice models and determing MOR expression as well as translating our results by using human studies via PET scans using an MOR agonist

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