The Role of *Zhx2* in Opioid-Induced, Naloxone-Precipitated Withdrawal Phenotypes

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Introduction

- **Oxycodone (OXY)** is a semi-synthetic narcotic analgesic and a major contributor to the 100,000+ drug-related deaths annually
- Opioid withdrawal symptom severity is one of the main factors driving relapse
- OXY is metabolized to **oxymorphone (OMOR)** via **cytochrome P450 (CYP450)** enzymes, a more potent version of the drug
- In the genetically similar **BALB/cJ** and **BALB/cByJ** strains of mice, we hypothesize that the transcriptional repressor protein **zinc finger homeobox 2 (Zhx2)** regulates CYP450 enzymes involved in OXY metabolism
- BALB/cJ express **MERV (mouse endogenous retroviral insertion)** which reduces Zhx2 expression withdrawal behavior
- **Our overall hypothesis** is that with the presence of MERV and subsequent decrease in Zhx2 expression, we will see an increase in metabolism of OXY to OMOR, and enhancement of withdrawal phenotypes

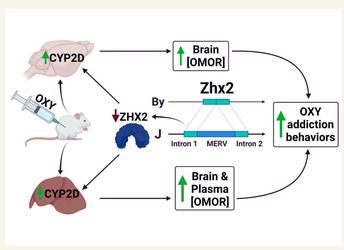
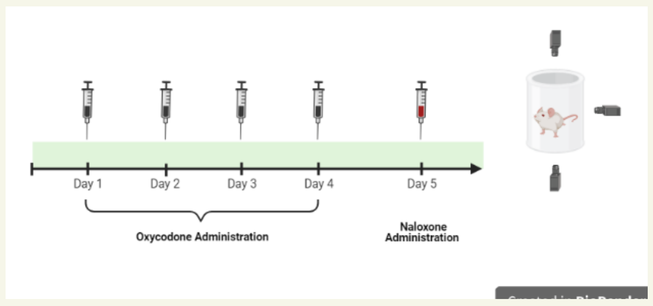


Illustration of Zhx2 involvement in OXY metabolism

Illustration of MERV insertion (red base pairs)

Methodology

- 72 mice are to be tested: 12/sex/genotype
- The CRISPR/Cas9-mediated mouse strains
  - Zhx2-MERV-KO (Removal of MERV)
  - Zhx2-Exon3-KO(Removal of Zhx2's only coding exon)
- Mice are injected with 40 mg/kg of OXY for 4 days to develop drug dependence
- On day 5, mice receive 40 mg/kg of OXY in the morning and 1mg/kg of **Naloxone** 4hrs later
- Withdrawal behavior will be recorded by 3 synchronized cameras



Protocol Timeline

Results

- Wildtype Zhx2-MERV-KO mice will express **exacerbated** withdrawal behavior compared to homozygotes who expressed no MERV.
  - E.g. Increased Grooming, Circling, Rearing
- Region-specific dissections in the brain will determine protein levels of Zhx2, further annotating its relationship to OXY metabolism and variations in withdrawal phenotypes
  - Anterior Cingulate Cortex
  - Ventral Tegmental Area
  - Nucleus Accumbens and the Reward Pathway
  - Periaqueductal Gray
  - Brain Stem

Preliminary quantification of withdrawal behaviors

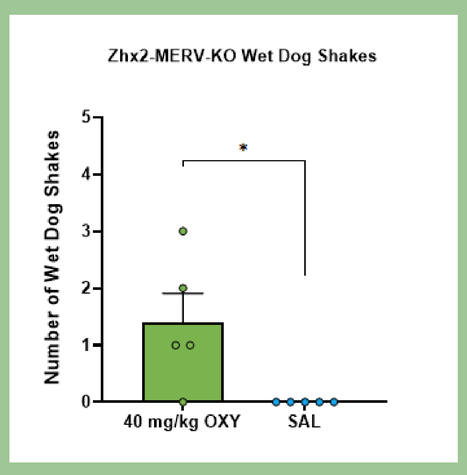


Figure 1. *Zhx2-MERV-KO mice show an increase in wet dog shakes during withdrawal following exposure to OXY compared to the saline controls.*
*(*p < 0.05)*

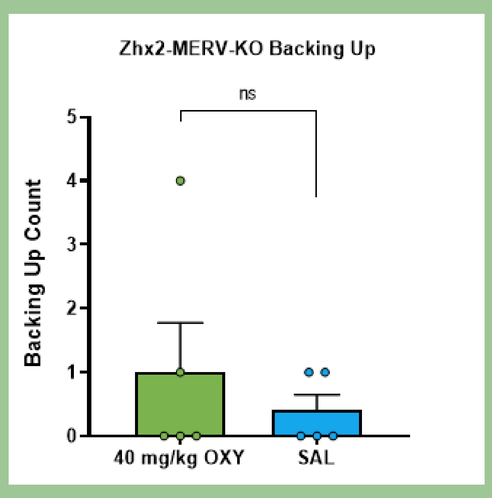


Figure 2. *Zhx2-MERV-KO mice show an upward trend in backing up during withdrawal after exposure to OXY compared to the saline controls. (p < 0.05)*

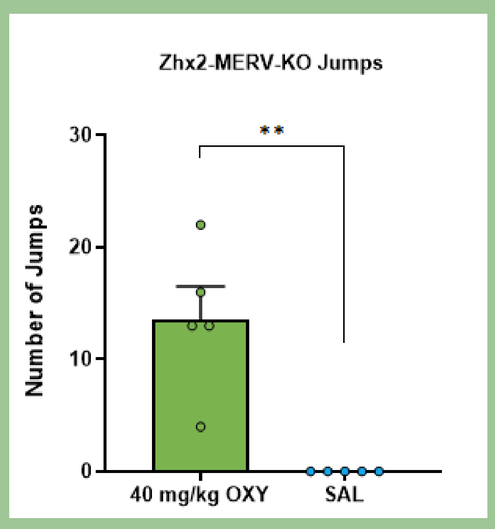
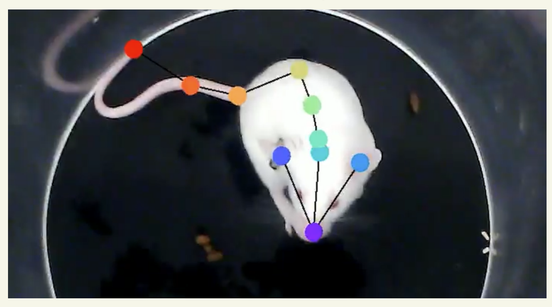


Figure 3. *Zhx2-MERV-KO mice show an increased number of jumps when exposed to OXY compared to the saline controls.* (**p<0.01)

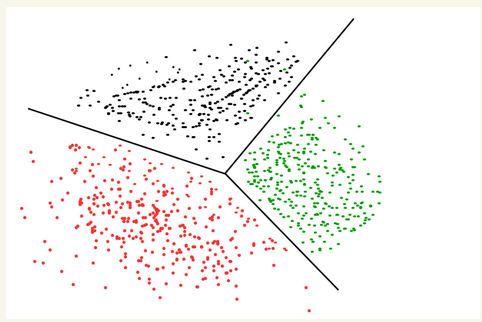
Conclusions

- With promising results from preliminary studies, our next steps are replicate these findings in a larger sample size, and implement machine learning identify subtle withdrawal behaviors (head oscillation, whisker movement, etc.)



Depiction of DeepLabCut-based tracking

- Computational analysis (dimensionality reduction, clustering, will be performed to further elucidate withdrawal phenotypic differences between genotypes



Depiction of Clustering

- Together, our research seeks to characterize withdrawal behavior phenotypes, and pinpoint causal genes involved in opioid use disorder.

References

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