Sex Differences in Morphine Withdrawal-Induced Anxiety: The Role of Estradiol and NMDA Receptors

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Introduction

- Opioid Use Disorder (OUD) affects millions worldwide, yet research on withdrawal has mostly focused on males.
- Men and women experience addiction and withdrawal differently, and understanding these differences can improve treatments.
- Estradiol (a key hormone in females) may impact anxiety during opioid withdrawal.

Objectives

1. Compare anxiety levels in male and female rats during opioid withdrawal.
2. Examine estradiol's role in withdrawal-related anxiety.
3. Study NMDA receptor changes in brain regions linked to anxiety and addiction.

Materials & Methods

Animals

- 29 adult Long Evans rats (Males: *N* = 17, Saline – *n* = 9, Morphine – *n* = 8; Females: *N* = 12, Saline – *n* = 6, Morphine – *n* = 6)

Lavage

- Saline flushed into the vaginal canal and cells are examined under microscope

Drugs and Dose

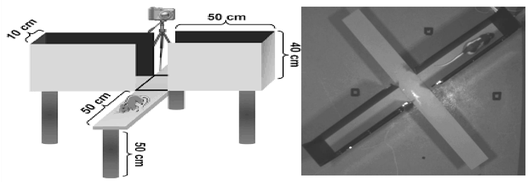
- Morphine sulfate in 0.9% saline – Injected s.c. twice daily for 10 days (escalating dose from 2.5 mg/kg to 40 mg/kg)
- Saline controls were injected with 0.1 mL/kg with 0.9% saline s.c.

Elevated Plus Maze (EPM)

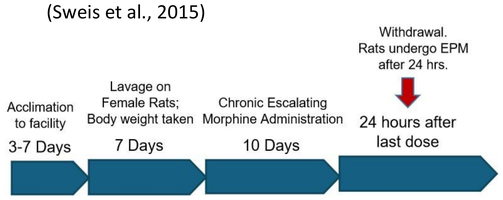
- Time spent and entries into open and closed arm - measures of anxiety-like behavior

Western Blot

- Anxiety: Basolateral Amygdala (BLA), Central Amygdala (CeA), Ventral hippocampus (Vhipp)
- Addiction: Nucleus Accumbens (NAc) core, NAc shell, Ventral Tegmental Area (VTA)

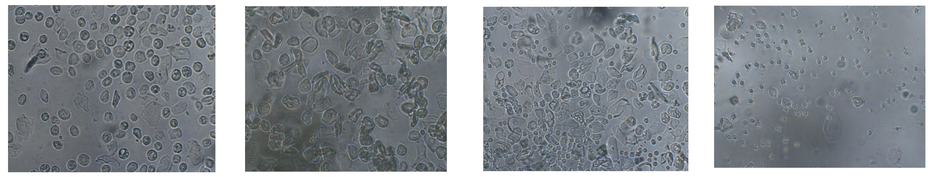


Elevated Plus Maze setup (Sweis et al., 2015)



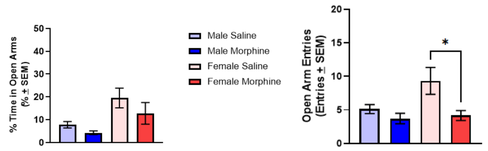
Materials & Methods cont.

Lavage Samples from each Stage of Estrous



Proestrus (high estradiol) | Estrus | Metestrus (low estradiol) | Diestrus

Results

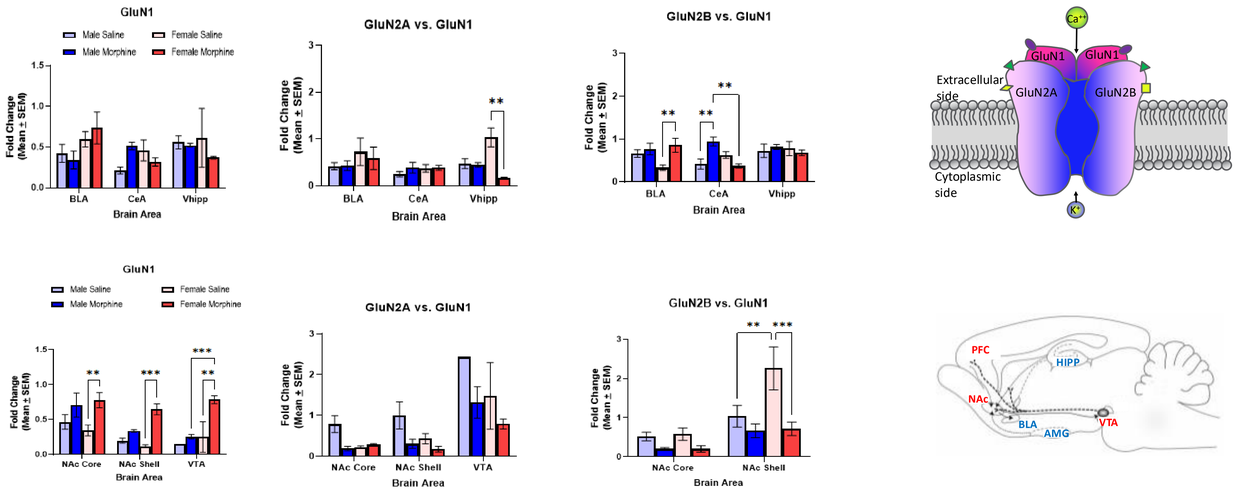


Anxiety-Like Behavior in the EPM by Sex

- Females generally spent more time in open arms (less anxiety) than males.
- After 24-hour morphine withdrawal, both sexes showed increased anxiety, but females exhibited more significant anxiety-like behavior.

Anxiety-Like Behavior in EPM by Hormone Condition

- High estradiol levels during withdrawal increased anxiety in females.
- Estradiol's role shifted from reducing anxiety (anxiolytic) to increasing anxiety (anxiogenic) during withdrawal.



* p < 0.05, ** p < 0.01, *** p < 0.001

Brain Region Differences in NMDA Receptor Subunit Expression

- Ventral Hippocampus (Vhipp): Females showed reduced GluN2A expression during withdrawal.
- Amygdala: Females had increased GluN2B expression in the basolateral amygdala (BLA), while males showed changes in the central amygdala (CeA).

Sex Differences

- Females: Showed increased GluN1 expression in the nucleus accumbens (NAc) after withdrawal, which may contribute to higher withdrawal sensitivity.
- Males: Had higher baseline levels of GluN2A-containing neurons in the NAc, potentially offering some protection during withdrawal.

Conclusions

Anxiety During Morphine Withdrawal:

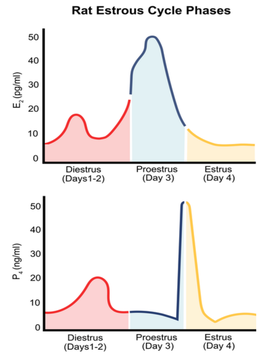
- Both male and female rats showed increased anxiety after morphine withdrawal, but males were generally more anxious.
- High estradiol (E2) levels increased anxiety in morphine withdrawn females.
- Estradiol's role shifted—normally reduces anxiety, but increased it during withdrawal.

Brain Changes in Morphine Withdrawal:

- Female rats had increased levels of a specific brain receptor (NMDA GluN1) in brain areas related to addiction, possibly making withdrawal more intense for them.
- There were differences between males and females in NMDA receptors in areas linked to anxiety (amygdala and hippocampus), showing the brain responds differently during withdrawal.

Why It Matters:

- These findings suggest estradiol influences withdrawal symptoms, which could explain why women experience stronger withdrawal distress.
- Understanding these sex differences could lead to better treatment strategies for opioid addiction.



Estradiol and progesterone levels during rat estrous cycle (Kokane & Perrotti, 2020)

Future Directions

- Ovariectomized (OVX) females
- Estradiol treatment with OVX females
- Western blot for effects of estradiol and ovariectomy on NMDA receptor expression
- Effects of other sex hormones (progesterone)

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