

Investigating sex differences in the motivational consequences of opioid withdrawal using conditioned place aversion paradigm in rats

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Background

- Opioid use disorder (OUD) is a chronic illness defined by cycles of intoxication, withdrawal, and relapse.
- Withdrawal induces negative physical and affective symptoms.
- Previous studies have shown that women demonstrate faster escalation of opioid use, and higher rates of craving and relapse.
- Lack of studies on sex differences on opioid withdrawal and its motivational influences.

Aims

- Investigate sex differences in the motivational consequences of opioid withdrawal using the conditioned place aversion paradigm.
- Identify differences in underlying neurobiological mechanisms

Methodology

Subjects

32 adult experimentally naïve intact male and female LE rats (m = 16, f = 16).

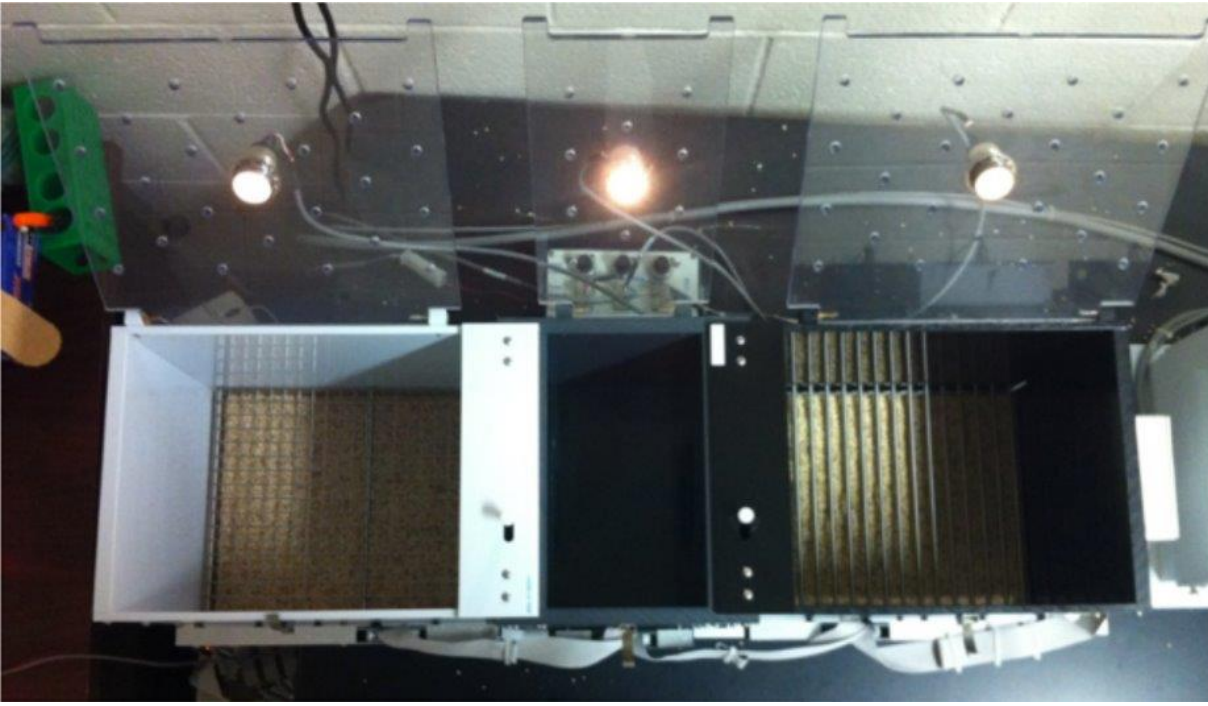
Drugs

Morphine sulfate (10 mg/kg; S.C.; Spectrum Chemical Manufacturing Corp.)
Naloxone (0.3 mg/kg; S.C.; Sigma-Aldrich)

Conditioned Place Aversion (CPA)

- Pretest (Day 1)– 15 minutes: rats allowed to freely explore the apparatus in a drug free state.
- Conditioning (Days 2–5)– 22 minutes: on alternating days, rats received saline injections and were confined to one chamber of the apparatus. The following day rats received a morphine injection followed by a naloxone injection 4 hours later to precipitate withdrawal and confined to the opposite chamber.
- Aversion Test (Day 6)– 15 minutes: rats freely explored apparatus.
- CPA = Posttest – Pretest

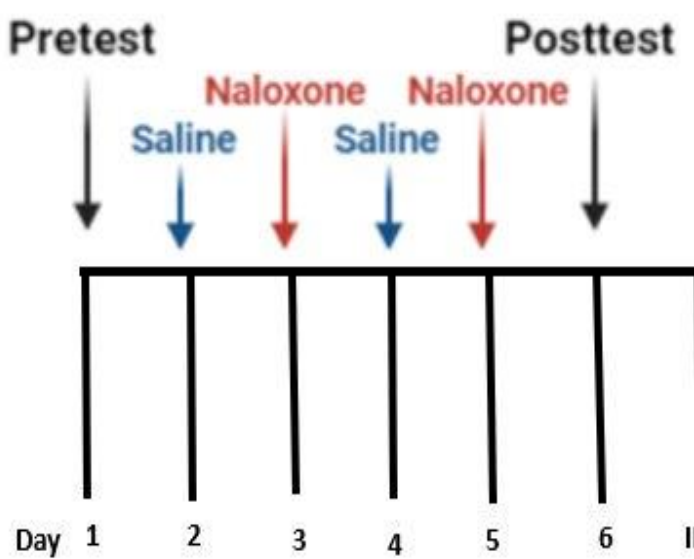
Figure 1. CPA apparatus & timeline of experimental procedure



Immunohistochemistry

- 15–20 mins after the post-test rats were transcardially perfused with 4% paraformaldehyde.
- Brains extracted
- Microtome sectioning – 40 µm coronal slices – mPFC, Nac, VTA.
- C-Fos (1/1000) immunoreactivity
- Cell counting using Gen5 – BioTek

Figure 2. Timeline of experimental procedure



Results

Figure 4. c-Fos expression in infralimbic cortex in intact males and females.

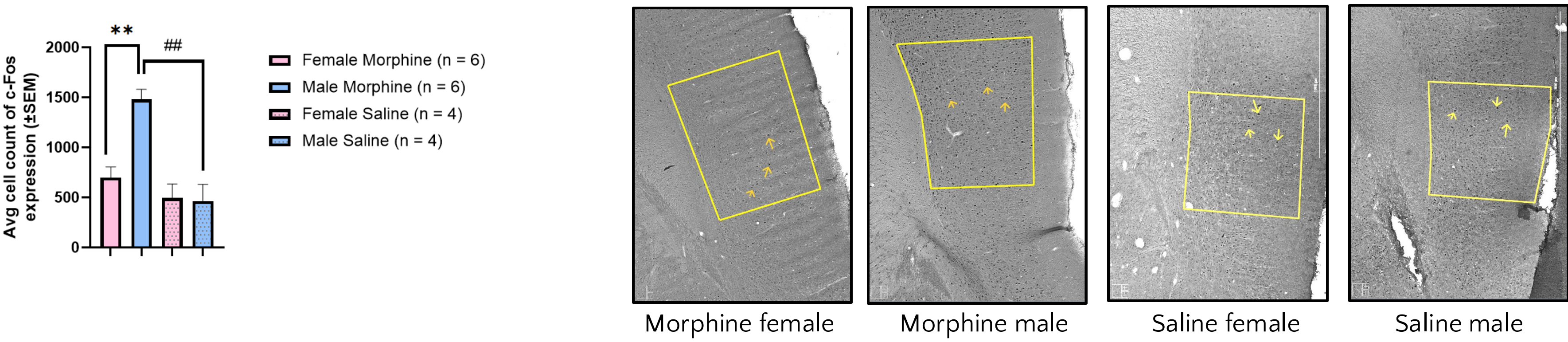


Figure 5. c-Fos expression in nucleus accumbens shell in intact males and females.

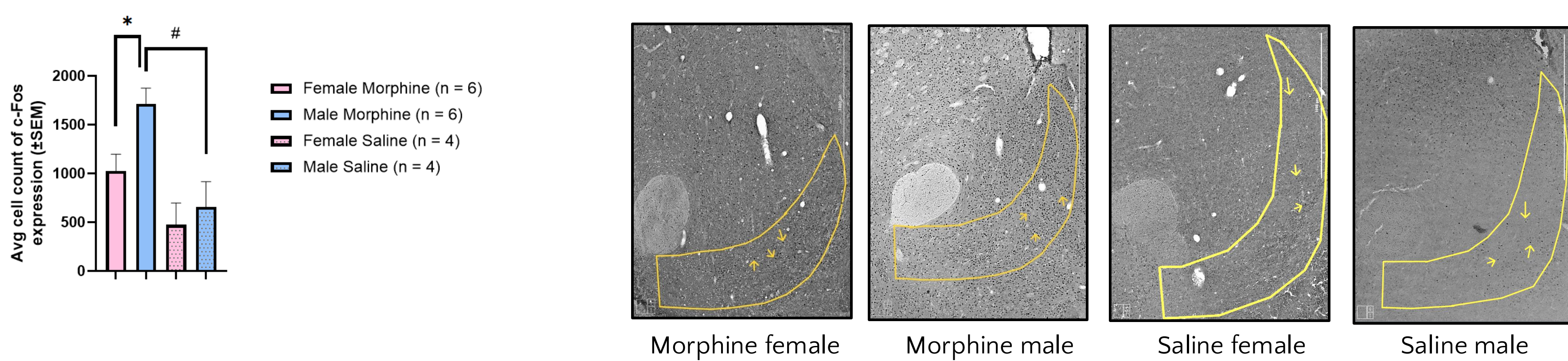


Figure 6. c-Fos expression in prelimbic cortex in intact males and females.

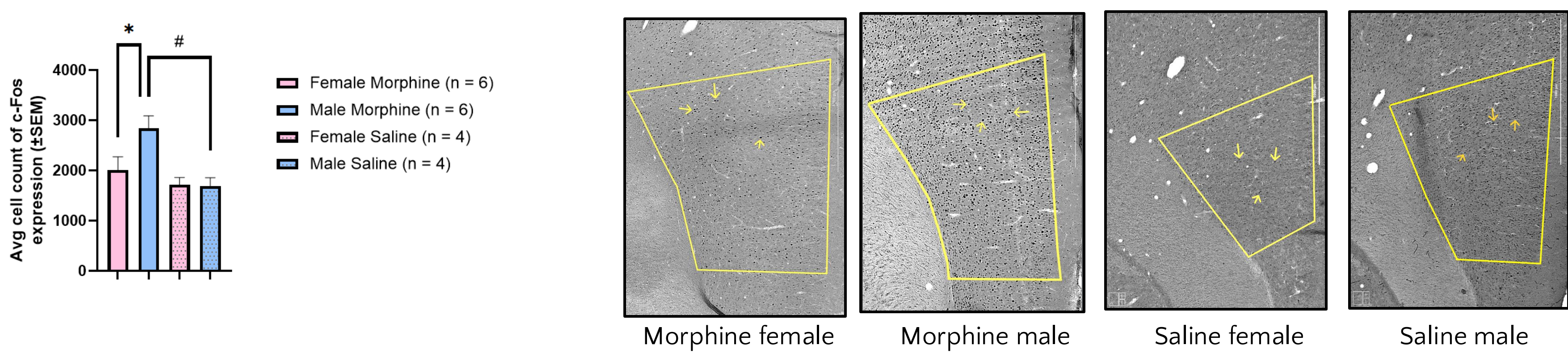


Figure 7. c-Fos expression in nucleus accumbens core in intact males and females.

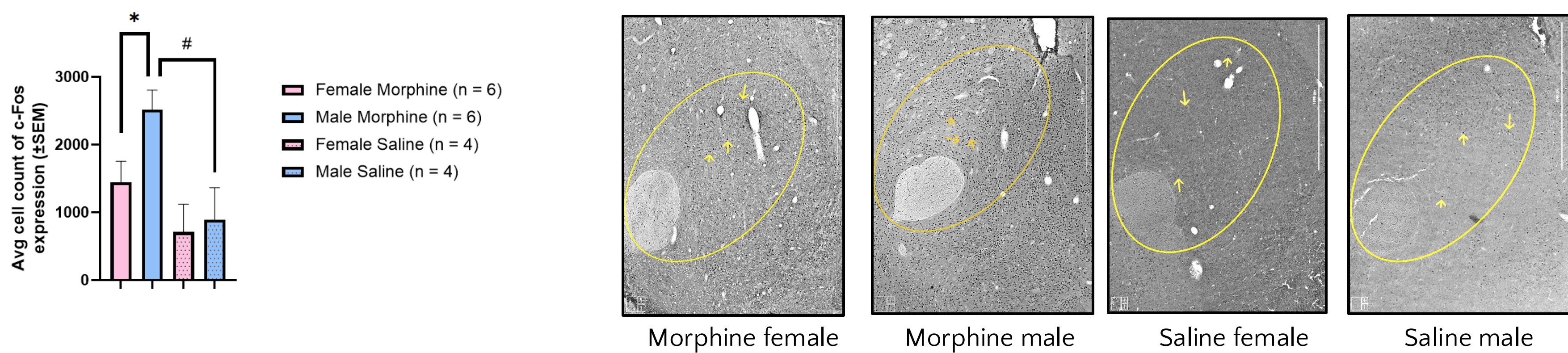
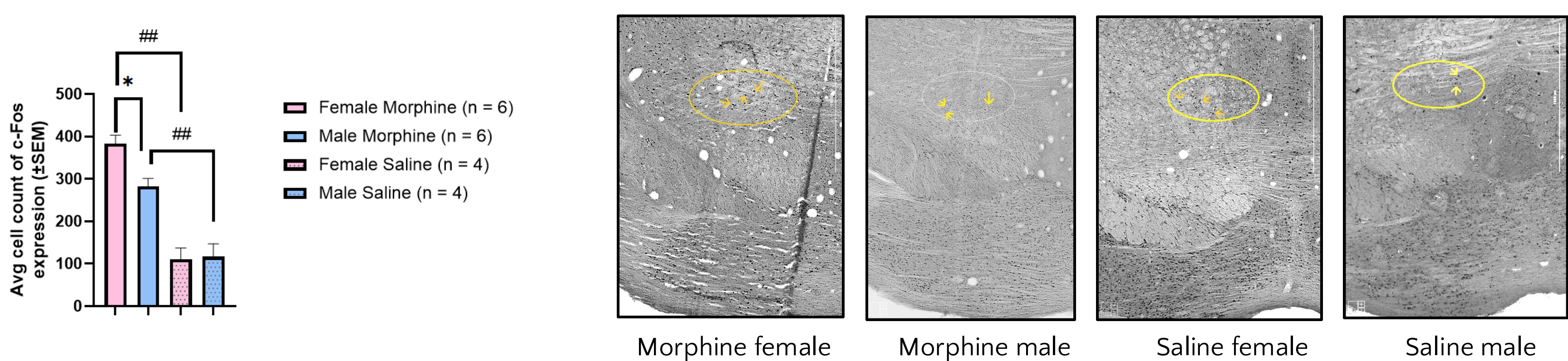
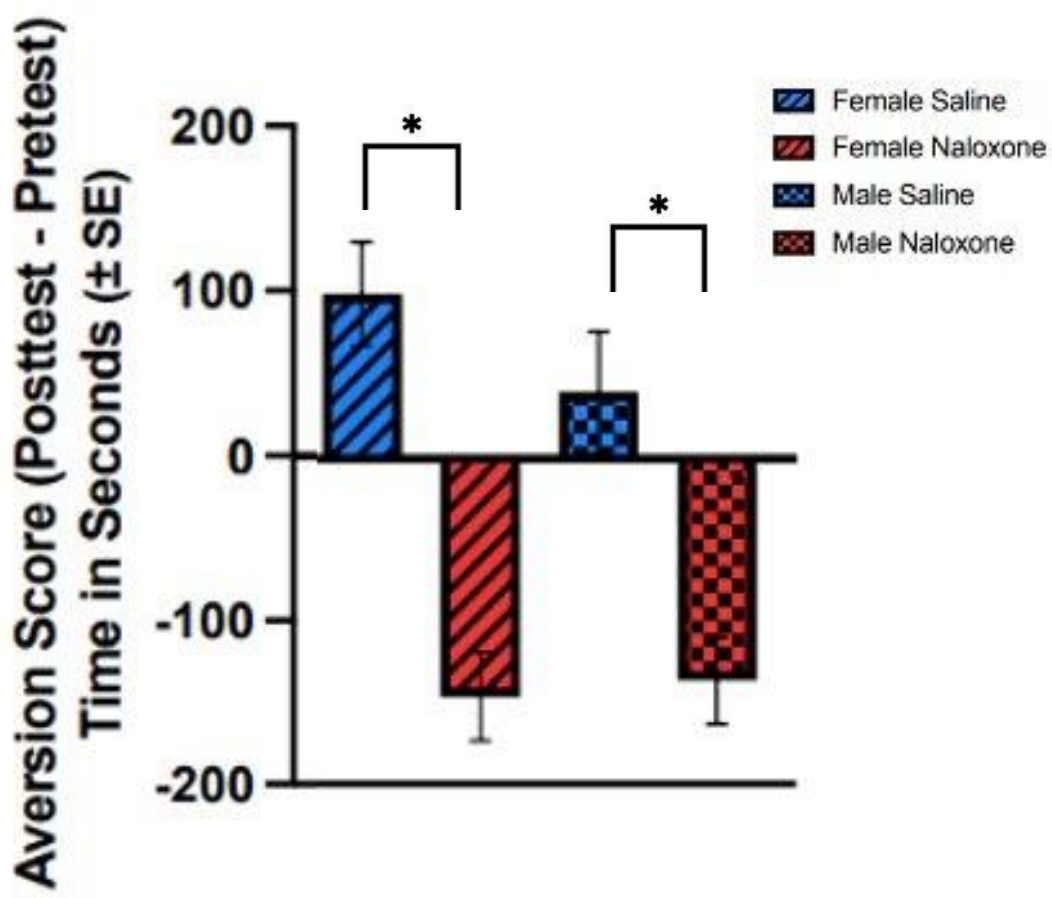


Figure 8. c-Fos expression in ventral tegmental area in intact males and females – morphine increased c-Fos in VTA of females



Results

Figure 3. CPA scores for intact males and females – no sex differences were seen in CPA scores between males (n = 12) and females (n = 12)



Summary

- Both males and females developed CPA after four days of conditioning.
- No sex differences were seen in aversion scores.
- In males, morphine increased c-Fos expression in all areas except VTA
- In females, morphine increased c-Fos expression in only VTA

Conclusion

- Sex differences were seen in activation of key nodes of reward pathway but not in CPA behavior.
- The data shown supports evidence of convergent sex differences in that behaviors exhibited are similar but the underlying biological processes differ.
- Increased c-Fos expression in IL cortex and Nacs suggests bidirectional activation of IL cortex to Nacs pathway.
- Additionally, increased c-Fos expression in PLC and Nacc suggests bidirectional activation of PLC to Nacc pathway.
- In males, activation of infralimbic → Nacs pathway and prelimbic → Nacc pathway shows enhanced associative learning. It was expected to see these pathways indicate higher c-Fos expression as previous literature suggests these pathways moderate aversive behavior. However, because these pathways were not as active in females, perhaps there is a different pathway that moderates aversion in females.
- Increased activation in VTA suggests dopaminergic neurotransmissions are enhancing the salience of the aversive environment.

Future Directions

- Examine c-Fos expression in paraventricular thalamus and locus coeruleus
- Chemogenetically inhibit specific pathways to isolate which pathway plays a role in CPA in males vs females

Acknowledgements

Research supported by NIDA/NIH under award number R15DA055201. Thank you to Saurabh Kokane, Ph.D. for his help and guidance throughout this project