

Kappa Opioid Receptor/Dynorphin Expression in Adult Female Mice with Adolescent Opioid Exposure and Adult Chronic Intermittent Ethanol Treatment

T. L. DuBose, L. L. Wilson, L. Albrechet-Souza, T. A. Wills

Louisiana State University Health Sciences Center, Department of Cell Biology and Anatomy, New Orleans, LA



Introduction

- Adolescence is a vulnerable period of brain development.
- Adolescent opioid exposure (AOE) results in elevated risk of prolonged opioid misuse (Windisch et al., 2020).
- Chronic opioid use increases pain sensitivity (Higgins et al., 2019).
- Alcohol use and withdrawal can induce hyperalgesia (You, 2017).
- The bed nucleus stria terminalis (BNST) is a sexually dimorphic brain region involved substance use disorders and, recently, pain.
- The oval and dorsolateral regions of the BNST are involved in the processing and regulation of negative affect and stress.
- The dynorphin/kappa opioid receptor (KOR) system is an important factor in BNST stress signaling also involved in addictive behaviors and pain.

Objective: This experiment investigated the relationship between mechanical and heat sensitivity in female mice with combined AOE and adult intermittent ethanol vapor exposure (CIE) and the activation of dynorphin and KOR in the BNST.

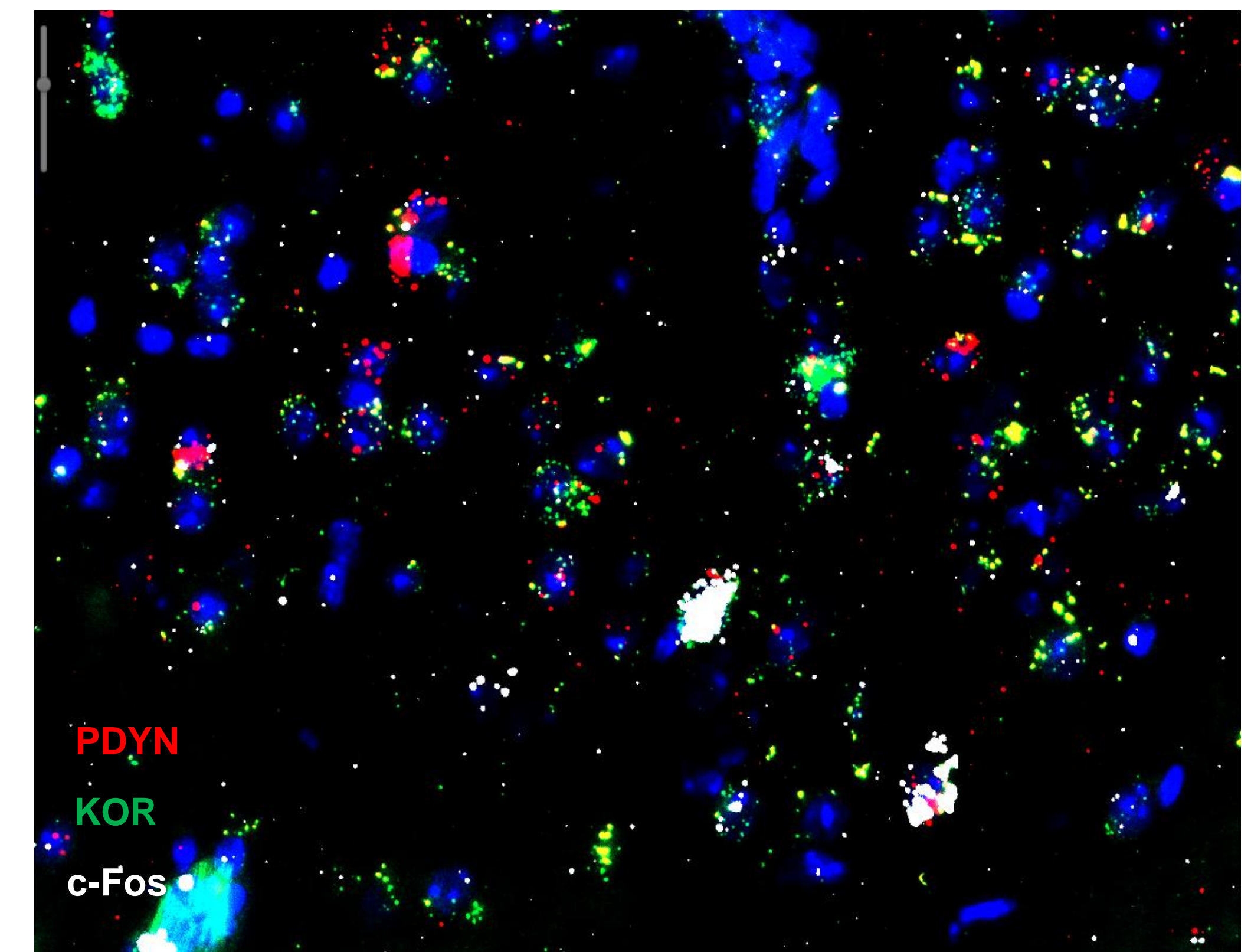
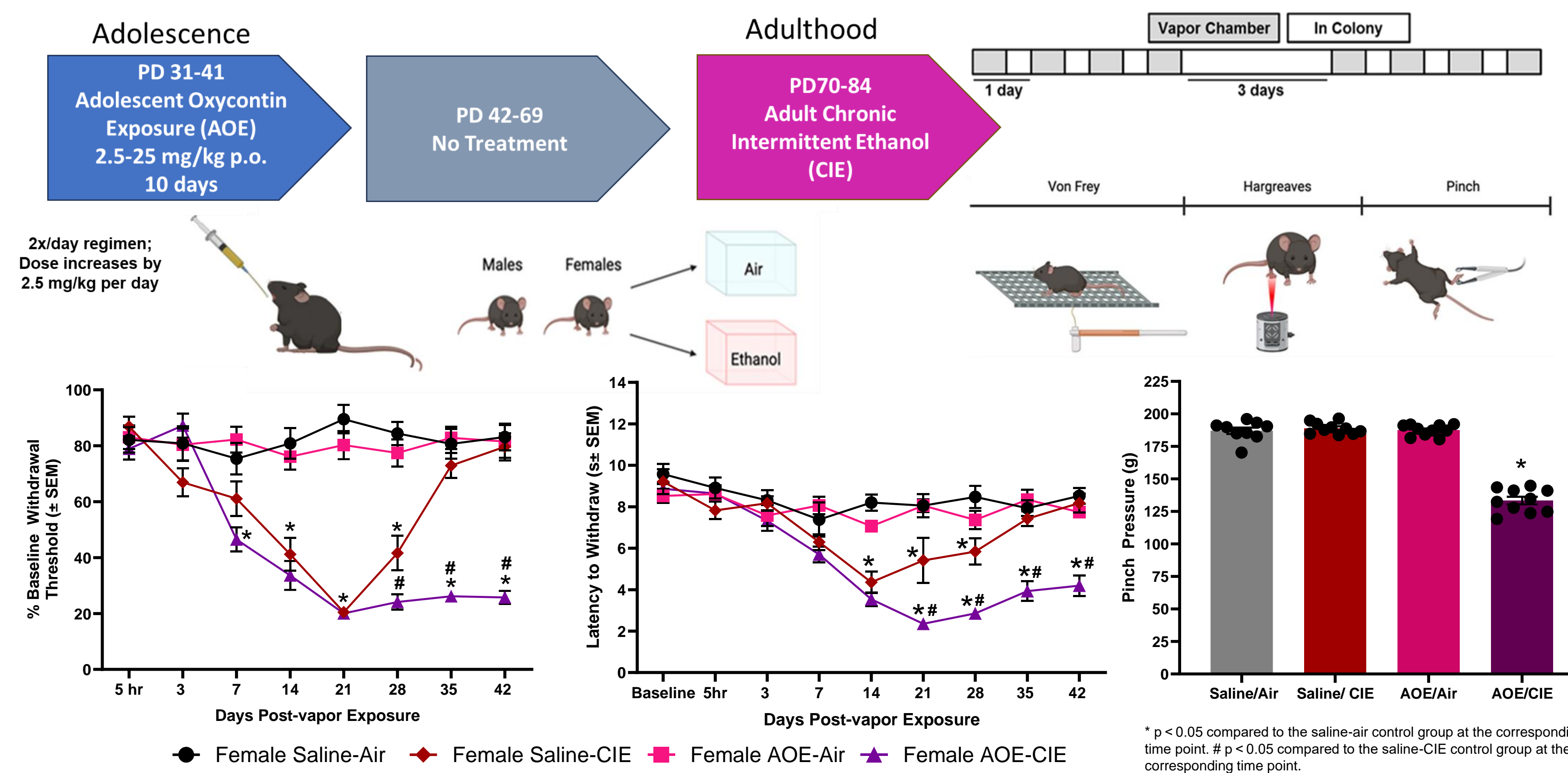
Methods

Adolescent Opioid Exposure (AOE) and Adult Chronic Intermittent Ethanol Treatment: Adolescent female C57BL/6J mice were injected with saline or 2.5-25 mg/kg oxycodone per day during postnatal days (PND) 31-42. Beginning PND 70 the mice were exposed to ethanol or water vapor chambers, undergoing two 4-day cycles of 16-h vapor exposure per day, separated by 3 days of no exposure.

Behavioral testing: Von Frey, Hargreaves, and pinch testing occurred weekly for 6 weeks to detect hyperalgesia in mice. Mechanical and heat sensitivity was assessed at 5 h, 3-, 7-, 14-, 21-, 28-, 35-, and 42 days post-vapor exposure. Acute reflexive pain was assessed 44 days post-vapor exposure.

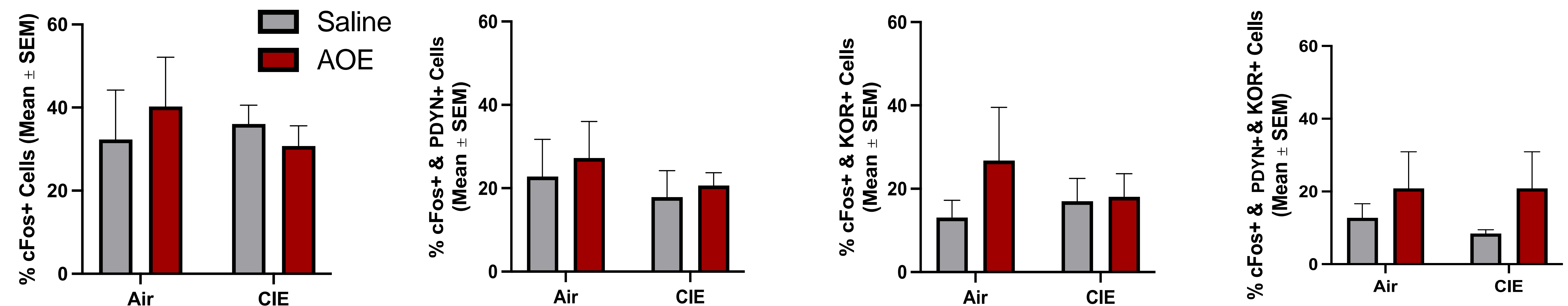
RNAscope: After mice brains were collected, they were flash frozen in isopentane and stored in -80°C until slicing. In-situ hybridization was performed on slides containing tissue from the BNST using the RNAscope Kit V2 with TSA Vivid Dyes. Nuclei were stained using DAPI and the following probes were used: c-Fos mRNA, a marker for cell activation; dynorphin mRNA (PDYN), a neuropeptide associated with pain and addiction; and kappa-opioid receptors mRNA (KOR), which are activated by PDYN cells. Slides were analyzed on the QuPath 0.4.3 software to automatically detect cells containing puncta. Only the positive expression of mRNA cells that met a threshold requirement of 5 were considered in the percentages expressed in the data.

Results

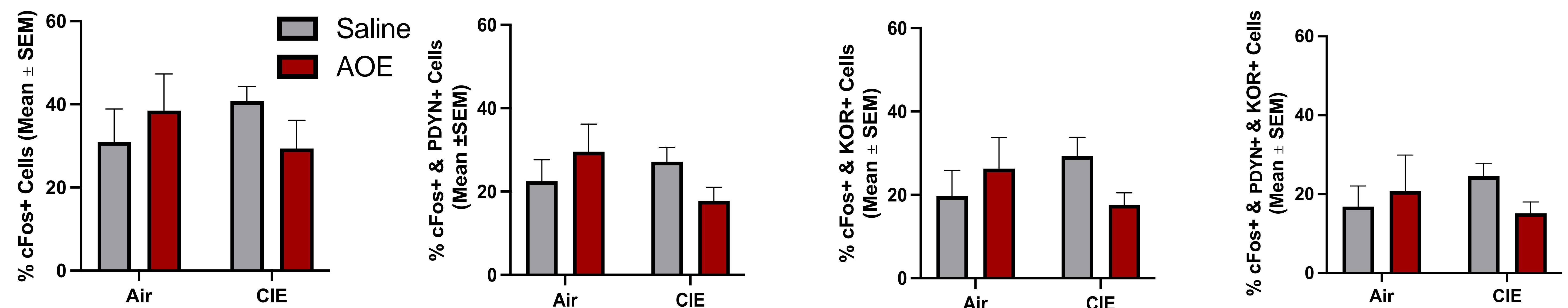


Dorsolateral BNST— Saline/Air

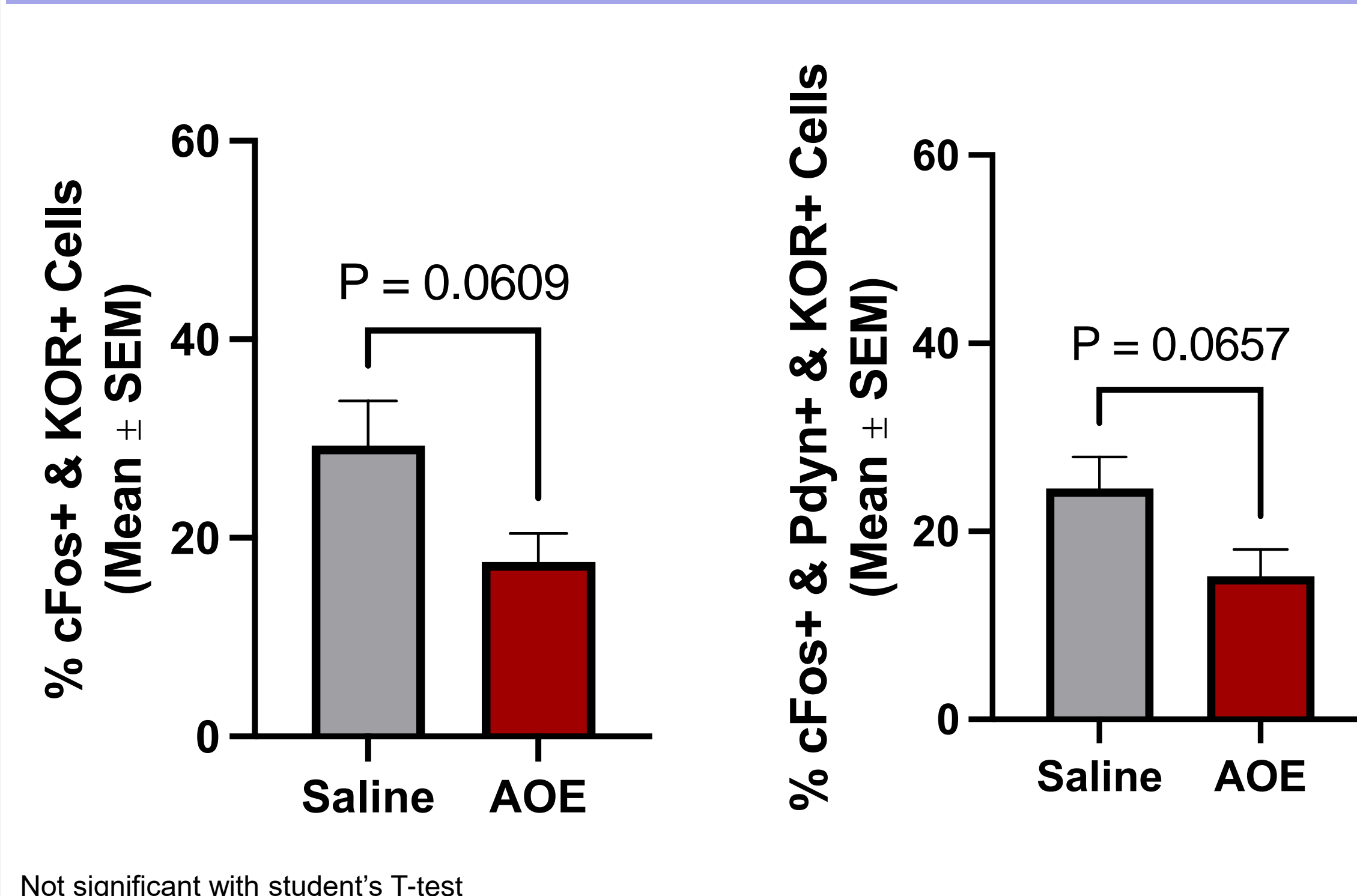
Oval BNST



Dorsolateral BNST



CIE Dorsolateral BNST



Conclusion

- Combined adolescent opioid exposure (AOE) and adult intermittent ethanol vapor exposure (CIE) induce persistent mechanical, heat, and acute pain sensitivity in female mice.
- Combined AOE and CIE females have decreased co-expression of cFos with PDYN and KOR cells in the dorsolateral BNST compared to mice exposed to saline and chronic intermittent alcohol exposure.
- Future studies will replicate this work to increase the number of mice per group.

Kappa Opioid Receptor/Dynorphin Expression in Adult Female Mice with Adolescent Opioid Exposure and Adult Chronic Intermittent Ethanol Treatment

T. L. DuBose, L. L. Wilson, L. Albrechet-Souza, T. A. Wills

Louisiana State University Health Sciences Center, Department of Cell Biology and Anatomy, New Orleans, LA



Introduction

- Adolescence is a vulnerable period of brain development.
- Adolescent opioid exposure (AOE) results in elevated risk of prolonged opioid misuse (Windisch et al., 2020).
- Chronic opioid use increases pain sensitivity (Higgins et al., 2019).
- Alcohol use and withdrawal can induce hyperalgesia (You, 2017).
- The bed nucleus stria terminalis (BNST) is a sexually dimorphic brain region involved substance use disorders and, recently, pain.
- The oval and dorsolateral regions of the BNST are involved in the processing and regulation of negative affect and stress.
- The dynorphin/kappa opioid receptor (KOR) system is an important factor in BNST stress signaling also involved in addictive behaviors and pain.

Objective: This experiment investigated the relationship between mechanical and heat sensitivity in female mice with combined AOE and adult intermittent ethanol vapor exposure (CIE) and the activation of dynorphin and KOR in the BNST.

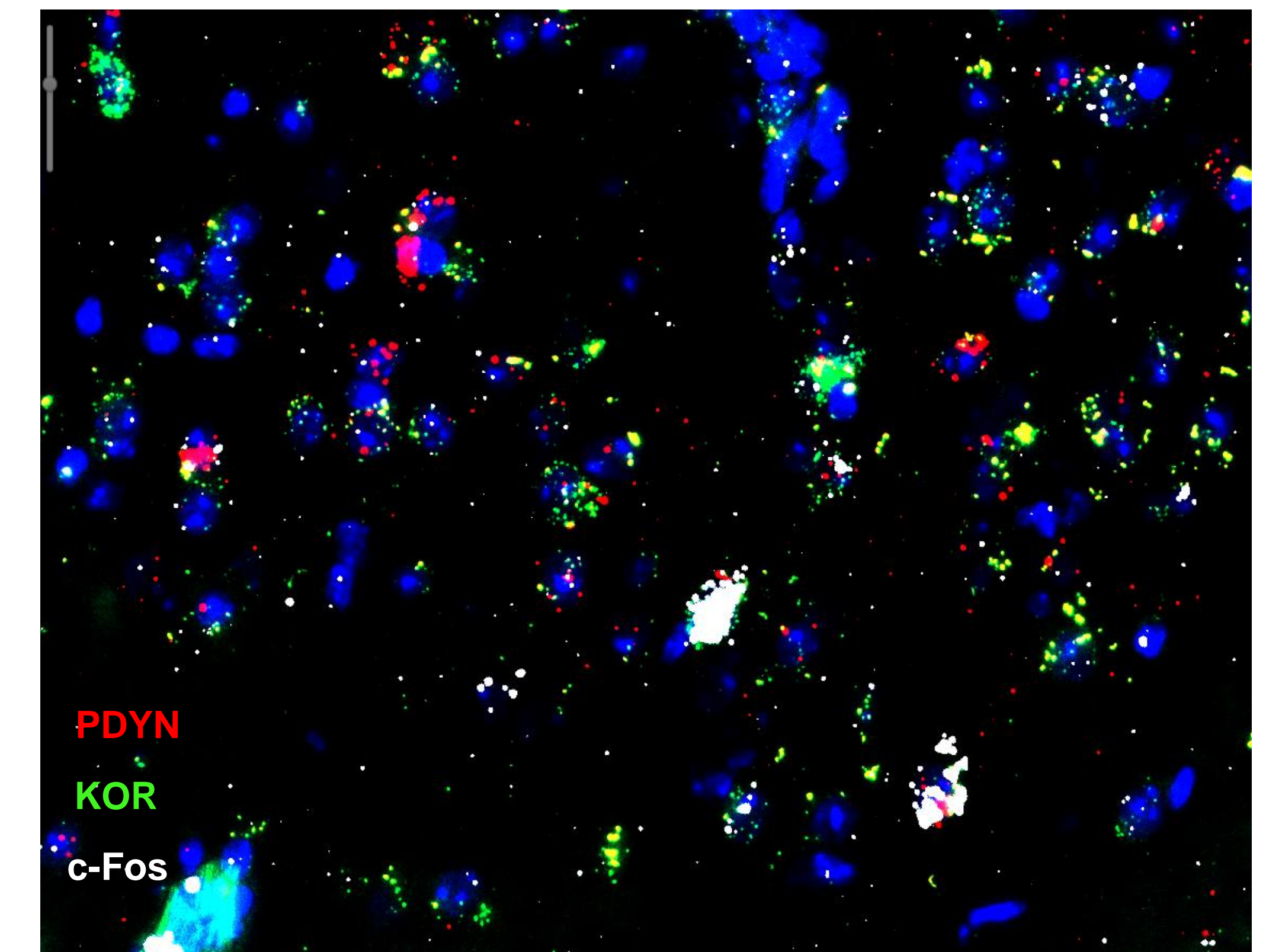
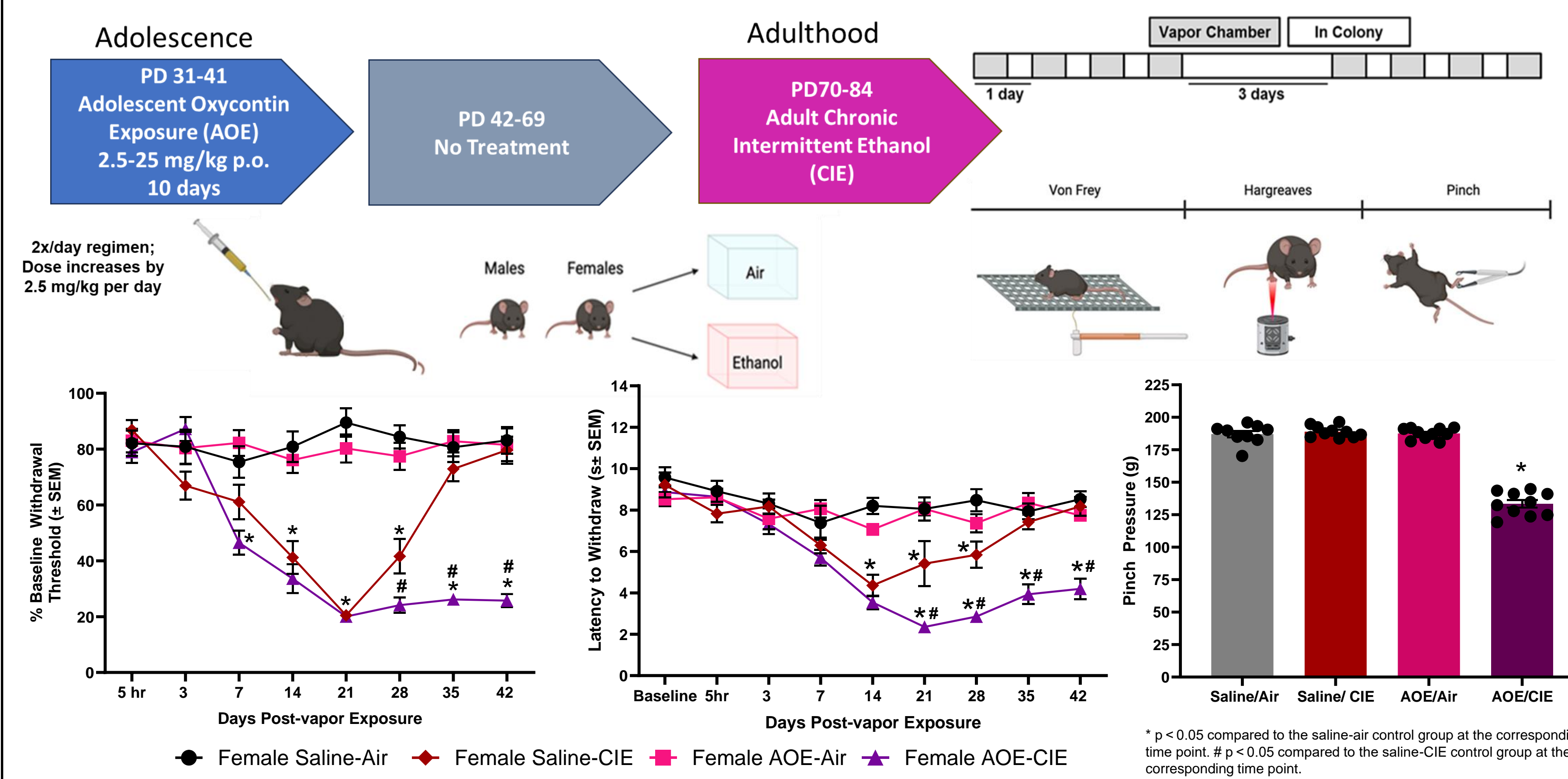
Methods

Adolescent Opioid Exposure (AOE) and Adult Chronic Intermittent Ethanol Treatment: Adolescent female C57BL/6J mice were injected with saline or 2.5-25 mg/kg oxycodone per day during postnatal days (PND) 31-42. Beginning PND 70 the mice were exposed to ethanol or water vapor chambers, undergoing two 4-day cycles of 16-h vapor exposure per day, separated by 3 days of no exposure.

Behavioral testing: Von Frey, Hargreaves, and pinch testing occurred weekly for 6 weeks to detect hyperalgesia in mice. Mechanical and heat sensitivity was assessed at 5 h, 3-, 7-, 14-, 21-, 28-, 35-, and 42 days post-vapor exposure. Acute reflexive pain was assessed 44 days post-vapor exposure.

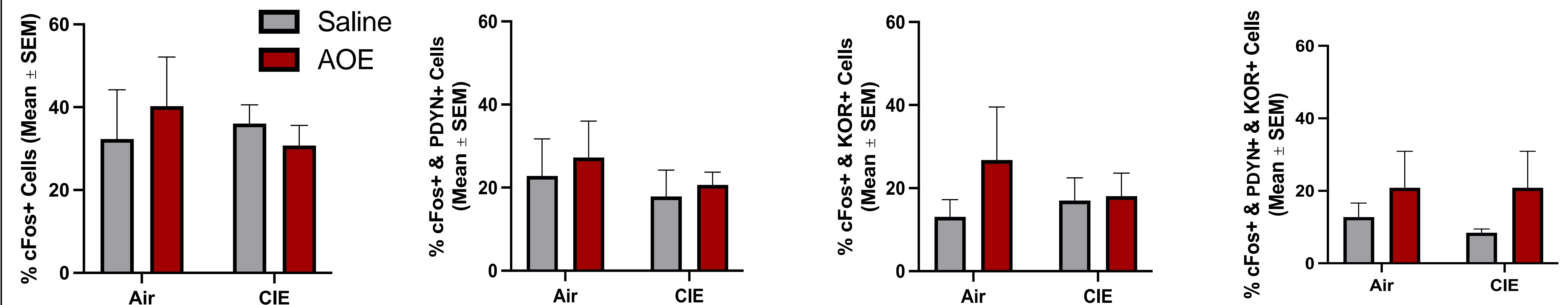
RNAscope: After mice brains were collected, they were flash frozen in isopentane and stored in -80°C until slicing. In-situ hybridization was performed on slides containing tissue from the BNST using the RNAscope Kit V2 with TSA Vivid Dyes. Nuclei were stained using DAPI and the following probes were used: c-Fos mRNA, a marker for cell activation; dynorphin mRNA (PDYN), a neuropeptide associated with pain and addiction; and kappa-opioid receptors mRNA (KOR), which are activated by PDYN cells. Slides were analyzed on the QuPath 0.4.3 software to automatically detect cells containing puncta. Only the positive expression of mRNA cells that met a threshold requirement of 5 were considered in the percentages expressed in the data.

Results

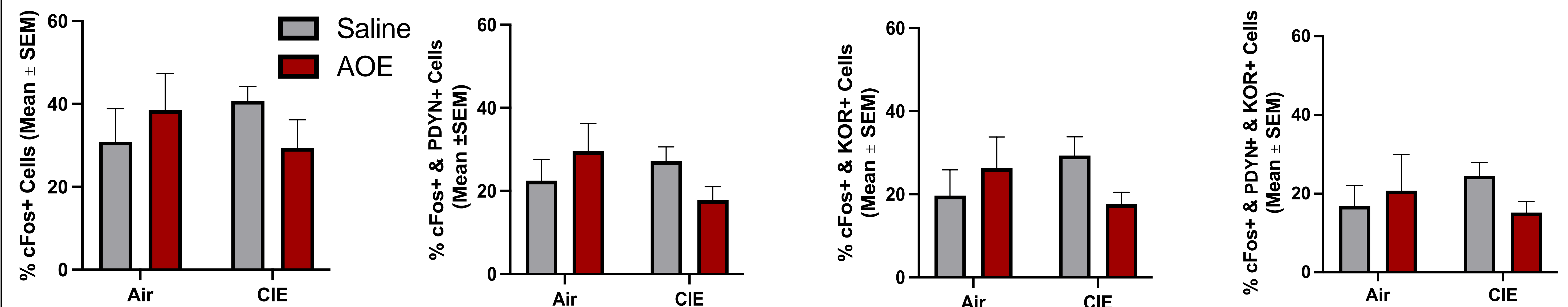


Dorsolateral BNST— Saline/Air

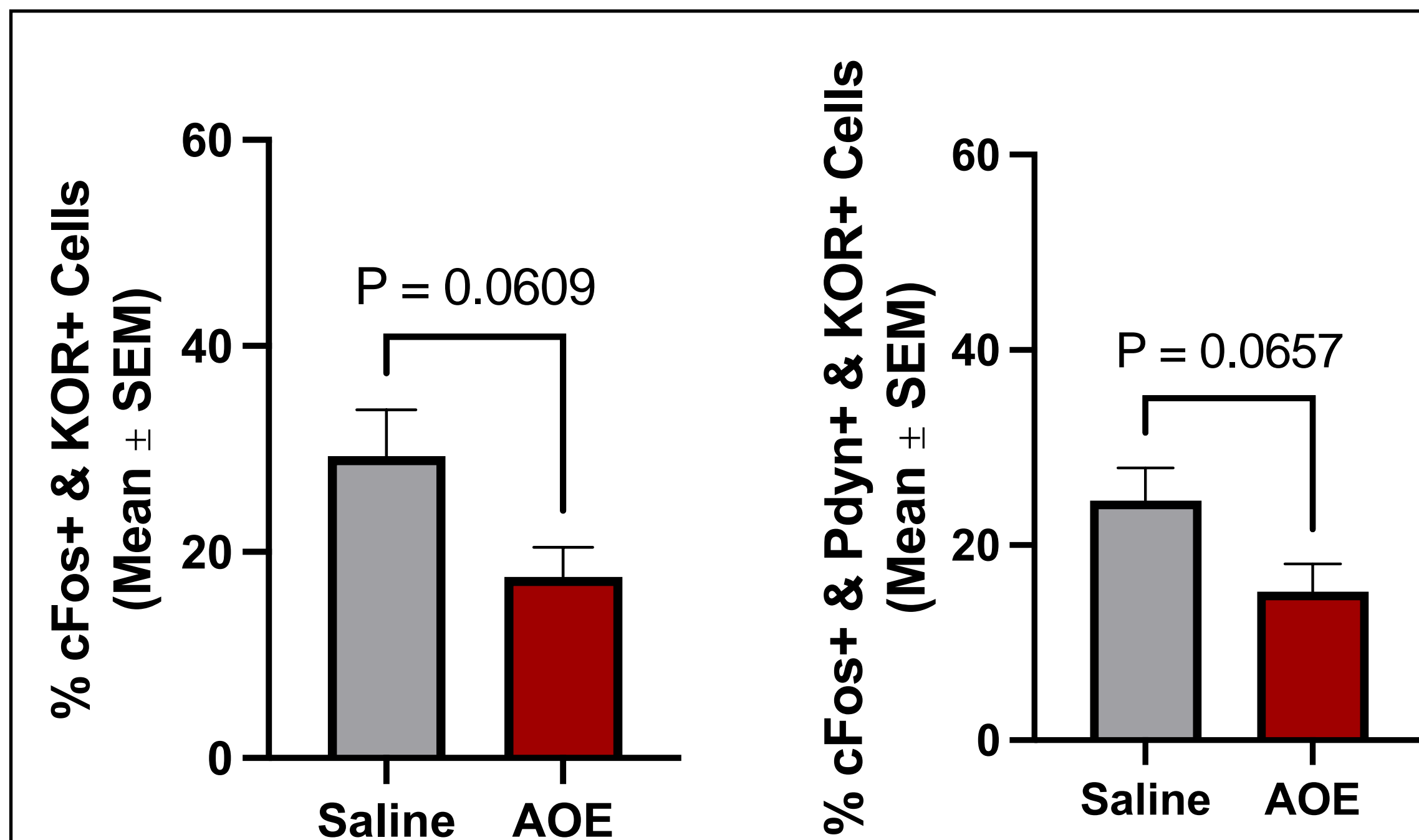
Oval BNST



Dorsolateral BNST



CIE Dorsolateral BNST



Not significant with student's T-test

Conclusion

- Combined adolescent opioid exposure (AOE) and adult intermittent ethanol vapor exposure (CIE) induce persistent mechanical, heat, and acute pain sensitivity in female mice.
- Combined AOE and CIE females have decreased co-expression of cFos with PDYN and KOR cells in the dorsolateral BNST compared to mice exposed to saline and chronic intermittent alcohol exposure.
- Future studies will replicate this work to increase the number of mice per group.