Lasting Neurobiological Impacts of Combined THC and Alcohol Use During Adolescence

T. MUSKU[1], B. AXE[1], D. ATHREYA[1], T. NELSON[1], S. BALAJI[1], A. MOORE[1], A. BRETTLER[1], S. SHAH[1], A. PHADKE[1], R. J. ORTIZ[2], P. KULKARNI[1], C. F. FERRIS[1]

[1]Northeastern Univ., Boston, MA; [2]Northern Illinois Univ., DeKalb, IL

Northeastern University

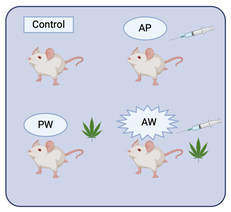
Background

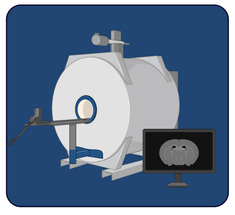
Simultaneous use of alcohol and cannabis during adolescence is rising, yet the long-term neurobiological consequences of this combined exposure remain poorly understood. During adolescence, the brain undergoes a critical period of neuroplasticity, making it more susceptible to developmental dysfunction. In this study, we examined how exposure to psychoactive substances elicits this change at the preclinical level, altering brain structure, functional connectivity, and executive behavior in adulthood through repeated co-exposure to Δ9-tetrahydrocannabinol (THC) and ethanol.

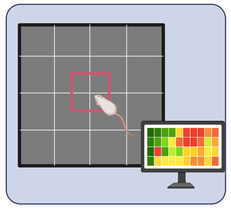
Methods

<u>Alcohol and Cannabis Exposure</u>

- **Day 0**: Adolescent male/female mice (PND 50): (N = 48, sex split) assigned to groups: PP (Double placebo), PW (Cannabis), AP (Alcohol), AW (Polysubstance)
- **Day 1-10**: Mice exposed to substance appropriate to group for ~ 10 day period. Days 1 and 10 followed by Open Field Test: exploratory locomotive behavior
  - <u>Alcohol Treatment</u>: Mice received either ethanol (4 mg/kg) or saline placebo via oral gavage
  - <u>Cannabis Treatment</u>: Mice placed in box → vaporization apparatus release 10.3% THC (.475g) or placebo for 2 mins → stop exposure → let sit in box for 20 additional mins







<u>Structural Neuroimaging</u>

- **Day 50-52:** 7.0 T Magnetic Resonance *Diffusion Weighted Imaging (DWI)*, EPI Sequence

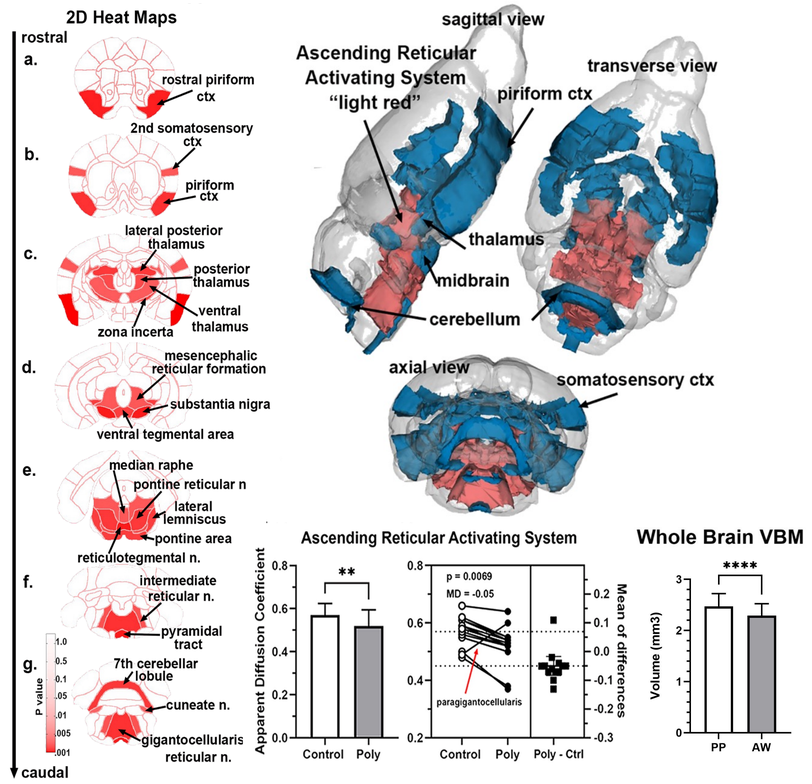
<u>Behavior Assays</u>

- **Day 52-54:** Open Field Test: exploratory locomotive behavior
- **Day 54-56:** Novel Object Recognition short-term memory

<u>Functional Neuroimaging</u>

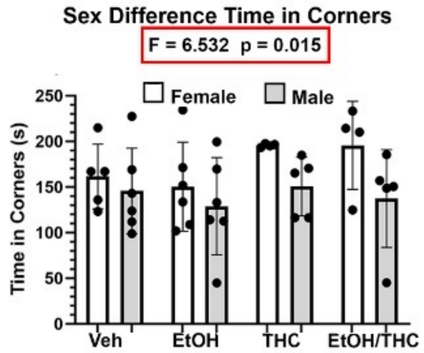
- **Day 56-60:** *T2 Weighted Anatomy*, *functional Magnetic Resonance Imaging* (fMRI), *Resting State Functional Connectivity* (rsFC), 7.0 T Magnetic Resonance

Gray Matter Microstructure



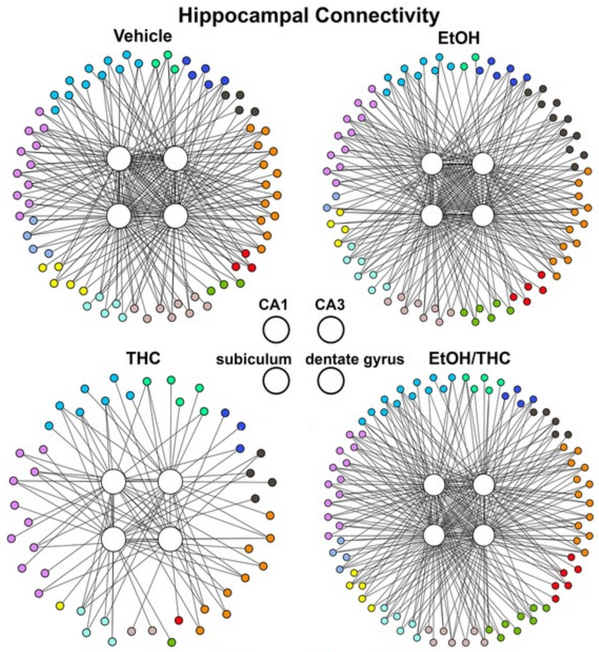
- AW group experienced reduced ADC values in the whole brain as well as several important brain regions, such as ARAS, basal ganglia, substantia nigra, VTA, and piriform cortex, indicating potential cytotoxic edema.
- Whole brain volume significantly reduced in the AW group
- AP males group experienced greater volume reductions compared to females
- AW females experienced greater volume reductions compared to males

Locomotor Behavior



A 2x2 ANOVA was conducted, yielding sex-dependent differences within THC and EtOH/THC groups. In females, increased time spent in corners may indicate heightened anxiety-like behavior.

Functional Connectivity



- Co-exposure to EtOH & THC drives a significant increase in whole-brain and regional connectivity compared to controls and single-substance groups.
- Exposure to THC alone blunts global and regional connectivity by over 50%.

Conclusion

Co-exposure to ethanol and THC during adolescence result in reduced overall brain volume and significant changes in brain activity in later developmental stages, particularly in regions pertaining to stress response and reward activation. Sex-specific differences were apparent, namely in short-term memory and behavior, implying the need to establish sex-tailored prevention strategies for polysubstance abuse going forward. fMRI was also conducted, though results did not yield statistical significance. Some examples of future directions this project may take are:

- Histology, metabolomics, and lipidomics studies
- Further sample size to increase power analysis

Acknowledgments

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