Using a Mouse Model to Explore the Neuroprotective Potential of Cannabidiol

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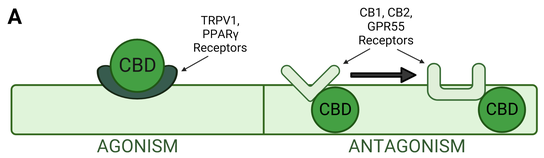
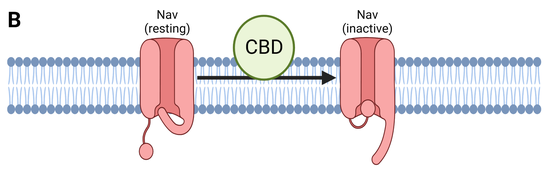
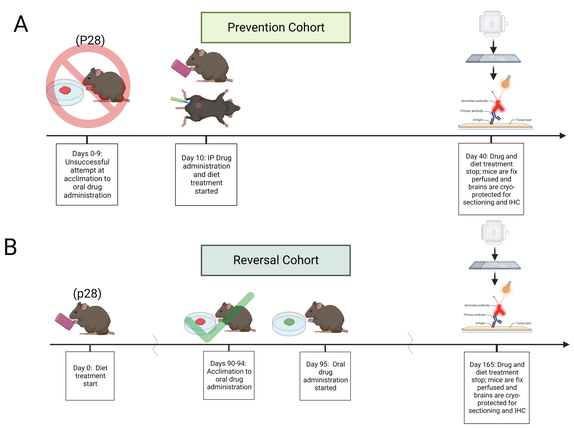
INTRODUCTION

As the medicalization of marijuana occurs across the U.S., the main non-psychoactive component, **cannabidiol** (CBD), has gained recognition for its therapeutic potential. Studies have explored the therapeutic ability of CBD in the treatment of certain neurodegenerative diseases or traumatic brain injury. We and others have reported a link between fatty diet consumption and increased neuronal loss and neuroinflammation in the olfactory and taste systems of mice. The objective for my honors project, therefore, was to assess the potential of CBD to mitigate neuroinflammation and neuronal loss induced by **diet-induced obesity** (DIO).





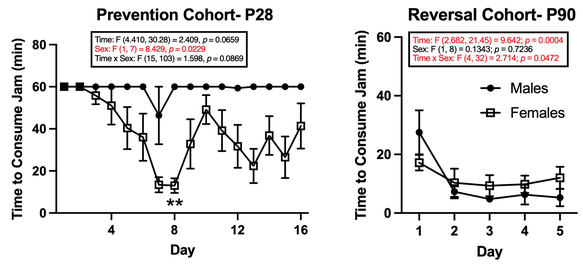
METHODS



Two Different Treatment Schemes =
A Prevention Cohort: Mice were simultaneously placed on a **moderately high-fat diet** (MHF; 32% kcal from fat) diet and administered CBD treatment to assess if CBD could prevent the deleterious effects of **diet-inducted obesity** (DIO). ***B Reversal Cohort:*** Mice were started on a MHF diet, and were placed on a CBD treatment 3 months later to assess if CBD could reverse the deleterious effects of the DIO. Both cohorts of mice were fix-perfused, and their brains were cryo-protected, sectioned, and immuno-labeled with anti-Iba-1, anti-Ki67, and anti-Caspase-3.

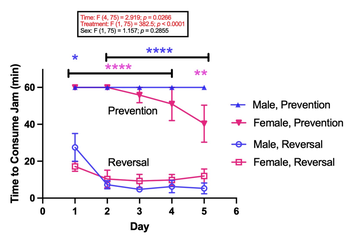
RESULTS

1. Early Postnatal Mice (P28) Had Difficulty to Learn Oral Jam Consumption Compared to that of P90 Adults



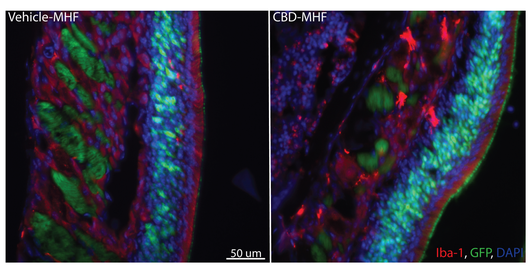
Jam acclimation curves for both male and female mice in the prevention cohort (left) and the reversal cohort (right). Prevention cohort data analyzed using a mixed effects analysis with time and sex as factors. Reversal cohort data analyzed using a 2-way repeated measures ANOVA with time and sex as factors and Sidak's multiple comparisons test. Data presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

2. Oral Jam Consumption Behavior was Sex Dependent in the Early Postnatal (P28) but not Adult Mice (P90)



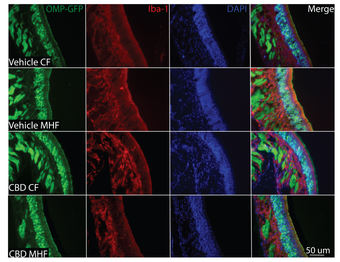
Jam consumption curves for days 1 to 5 for male and female mice in the prevention and reversal cohorts. Data analyzed using a three-way ANOVA with time, treatment, and sex as factors and Tukey's multiple comparisons test. Data presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

3. Markers of Inflammation and Olfactory Sensory Neurons (OSNs) Are Fluorescently Labeling in the Main Olfactory Epithelium in the Prevention Cohort Mice



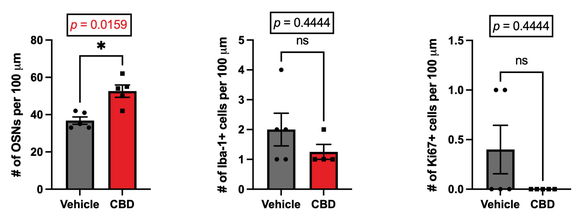
Representative images of a **moderately high-fat** (MHF) fed mouse that received vehicle (saline) injections (left) and a MHF fed mouse that received CBD injections (right). The **main olfactory epithelium** (MOE) of mice with a native **green fluorescent protein** (GFP) signal in mature **olfactory sensory neurons** (OSNs) were cryosectioned. 15 um thick sections were stained with an antibody for ionized calcium binding adaptor molecule 1 (Iba-1, 1:1,000) to visualize inflammatory cells (red). Tissue were also stained with **4',6-diamidino-2-phenylindole** (DAPI) in blue to visualize nuclei.

4. Markers of Inflammation and OSNs are Similarly Labeled in the Reversal Cohort Mice



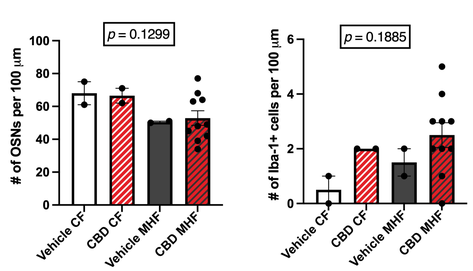
Representative images of mice across drug (vehicle or CBD) and diet (control fed (CF) or MHF) treatments. The MOE of mice with a native GFP signal in mature OSNs (called OMP*gfp* mice) were cryosectioned. 15 um thick sections were stained with an antibody for **ionized calcium binding adaptor molecule 1** (Iba-1, 1:1,000) to visualize inflammatory cells (red). Tissue was also stained with DAPI nuclear stain (blue).

5. CBD Increases the Number of OSNs in Mice Simultaneously Maintained on a MHF Diet



Quantification of mature OSNs (left), inflammatory cells (middle) and proliferating cells (right) in the MOE of mice treated with vehicle or CBD while maintained on a MHF diet. Data were analyzed with Mann-Whitney tests. Data presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

6. OSN Abundance and Inflammation Is Being Examined in Mice Pretreated on MHF Diet to Induce DIO and then Administered CBD



Quantification of mature OSNs (left) and inflammatory cells (right) in the MOE of mice treated with vehicle or CBD while maintained on either a control diet (CF) or a moderately high-fat (MHF) diet. OSN data were analyzed with a Brown-Forsythe ANOVA test with Dunnett's T3 multiple comparisons test. Iba-1 data were analyzed with a Kruskal-Wallis test with Dunn's multiple comparisons test. Data are represented as mean ± SEM.

CONCLUSIONS

1. Jam consumption is an age-related behavior. Early postnatal mice (P28) were unable to consistently learn the task while adult mice (P90) learned to consume jam from dishes within one minute of presentation.
2. The disability to learn oral jam consumption was sex dependent in younger (P28) mice, whereas males and females showed no difference in learning as adults (P90).
3. CBD prevented neuronal loss from occurring when mice were placed on a MHF for 30 days without creating any observable effect on inflammation or cell proliferation.
4. CBD has no observable effect on inflammation, cell proliferation, or OSN abundance in mice with DIO following 3 months of MHF diet maintenance.

REFERENCES

1. El Sohly MA, Radwan MM, Gul W, Chandra S, Galal A. Phytochemistry of Cannabis sativa. *Prog Chem Org Nat Prod.* 2017;103:1-36.
2. Gorzkiewicz, A., Szemraj, J., 2018. Brain endocannabinoid signaling exhibits remarkable complexity. *Brain Res Bull* 142, 33–46.
3. Hind, W.H., England, T.J., O'Sullivan, S.E., 2016. Cannabidiol protects an in vitro model of the blood-brain barrier from oxygen-glucose deprivation via PPARγ and 5-HT1A receptors. *Br J Pharmacol* 173, 815–825.
4. Patel, S., Hill, M.N., Cheer, J.F., Wotjak, C.T., Holmes, A., 2017. The endocannabinoid system as a target for novel anxiolytic drugs. *Neurosci Biobehav Rev* 76, 56–66.

ACKNOWLEDGEMENTS

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