



# Health and Behavioral Consequences of Prenatal Cannabidiol (CBD) Exposure

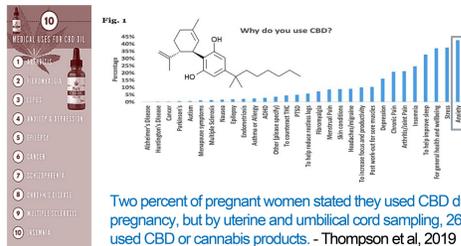


Martina Compagno<sup>1</sup>, Amber Bernstein<sup>2</sup>, Caroline Bishop<sup>2</sup>, Aidan Carley<sup>2</sup>, Josh Cazorla<sup>2</sup>, Jenna Claydon<sup>2</sup>, Alexis Cox<sup>2</sup>, Ashleigh Crane<sup>2</sup>, Chloe Crisp<sup>2</sup>, Emma Curley<sup>2</sup>, Ashley Loeven<sup>2</sup>, Camilla May<sup>2</sup>, Frank Pacheco<sup>2</sup>, Claudia Silver<sup>3</sup>, Olivia Turner<sup>2</sup>, and Debra Ann Fadool<sup>1, 2, 3</sup>

The Florida State University, <sup>1</sup>Institute of Molecular Biophysics, <sup>2</sup>Department of Biological Science, and <sup>3</sup>Program in Neuroscience

## INTRODUCTION

Cannabis has been used for centuries for its medicinal and psychoactive properties. **Cannabidiol (CBD)** is a non-psychoactive component of cannabis, and is used by the public to self-treat a myriad of ailments, including anxiety, depression, and inflammation, among others. CBD is only FDA-approved, however, for the treatment of Lenard-Gastaut-related epilepsy.

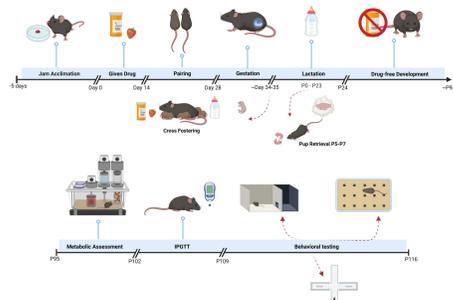


Two percent of pregnant women stated they used CBD during pregnancy, but by uterine and umbilical cord sampling, 26% had used CBD or cannabis products. - Thompson et al, 2019

Gestational CBD use is rising, particularly for treatment of pregnancy-related symptoms, including nausea, insomnia, and chronic pain. Our study utilized oral CBD administration, meant to mimic an edible route of delivery, and examined the health and behavioral consequences of perinatal CBD exposure.

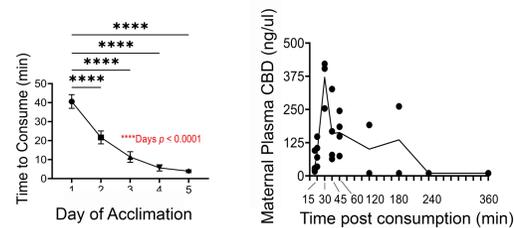
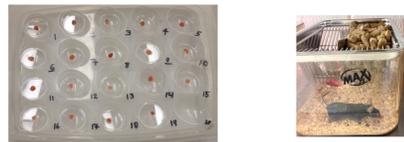
## METHODS

Primiparous female mice were acclimated to strawberry jam feeding for 5 days. Following acclimation, they were treated with 100 mg/kg CBD for 14 days prior to mating, and then drug administration continued as long as through weaning at 3 weeks of age. Upon giving birth, offspring were cross-fostered to separate influences of maternal behavior. Dams were evaluated for pregnancy outcomes and maternal behavior was tested via a pup retrieval test, whereas offspring were drug free upon weaning and examined as adults. At 3 months old, perinatally exposed offspring were metabolically assessed utilizing a **comprehensive laboratory animal monitoring system (CLAMS)** and an **intraperitoneal glucose tolerance test (IPGTT)**. Adult offspring completed a series of behavior tests, including marble-burying, **light-dark box (LDB)**, **elevated plus maze (EPM)**, and an object memory test, and an object attention task.



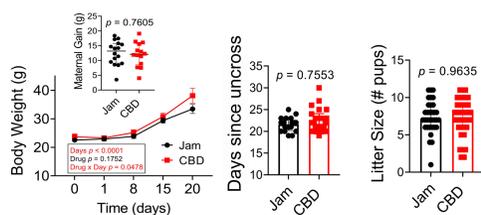
All mice in our study were housed in the **Florida State University (FSU) vivarium** with reverse 12/12-hr light/dark cycle (lights off at 9 am and on at 9 pm). Experiments were approved under protocol number #202000036 by the FSU **Institutional Animal Care and Use Committee (IACUC)**. Experiments were performed on approximately 3-month old male and female C57BL/6J mice. Behaviors were digitally recorded and manually analyzed post-hoc by investigators blinded to treatment condition. Representative traces were generated utilizing DeepLabCut and a custom R script.

## 1. Mice can be trained to orally consume CBD in strawberry jam.



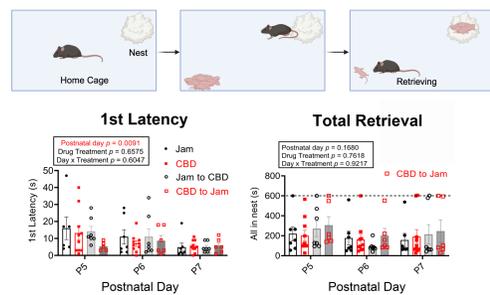
Mice can be acclimated to eating a 100 mg dollop of strawberry jam in a dish to provide an effective oral delivery of the drug in pregnant mice.

## 3. CBD does not affect maternal weight gain, gestation length, or litter size.



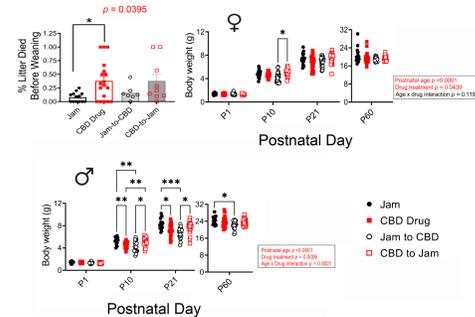
Oral CBD consumption has no significant effect on general measures of pregnancy and development, however, see panel 4.

## 3. Oral CBD administration does not affect pup retrieval by the dam.



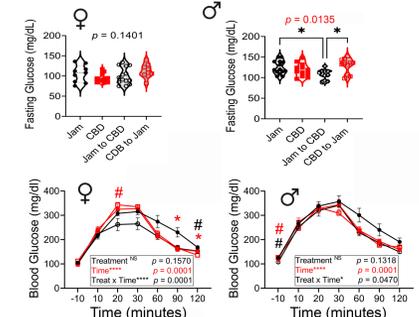
CBD does not significantly affect pup retrieval by the dam as a metric of maternal behavior. Dams were found to move more quickly toward their pups across days of behavioral testing, but no difference was found across treatment for latency in moving toward the first pup or in time to retrieve all pups.

## 4. Gestational CBD significantly decreases pup survival to weaning, and decreases early life body weight in males.



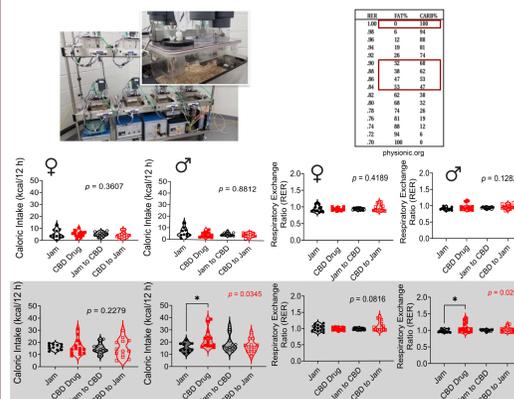
Gestational CBD exposure decreases pup survival to weaning age, where 40% of pups exposed to CBD died before weaning. Additionally, males exposed to CBD *in utero* had significantly decreased early life body weights, but this is helped when pups are cross-fostered to a drug-free dam.

## 5. Males exposed to CBD during lactation have lower fasting glucose.



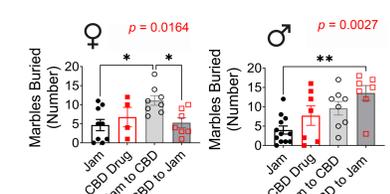
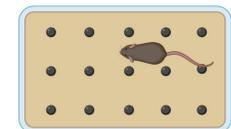
Males exposed to CBD during lactation have a lower fasting glucose, and females have a faster rate of glucose clearance, though this does not translate to change in glucose tolerance.

## 6. Perinatal CBD exposure causes sex-specific changes in ingestive behavior and fuel utilization.



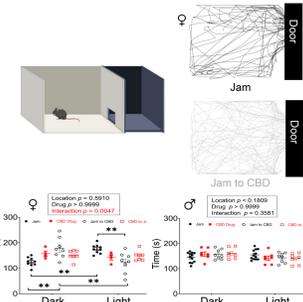
Perinatal CBD exposure causes an increase in caloric intake in adult male offspring in the dark cycle and this is accompanied by an increase in RER, respiratory exchange ratio or the Volume O<sub>2</sub> / Volume CO<sub>2</sub>, indicating an increased use of carbohydrates as fuel.

## 7. Early postnatal exposure to CBD increased marble-burying behavior.



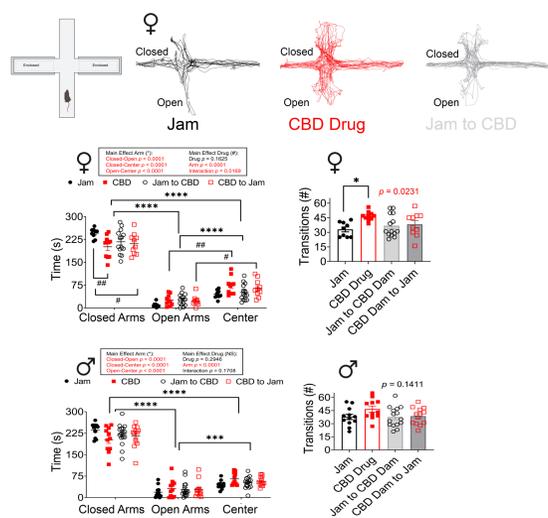
Early postnatal CBD increased marble burying (**obsessive compulsive-like behavior**) in adults, which could be reversed in females by cross-fostering to drug-free dams.

## 8. Early postnatal CBD increased resident time spent in the light for females in a light-dark box.



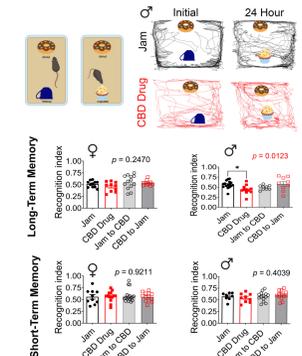
Early postnatal CBD exposure increased resident time spent in the light in adult females, indicating a decrease in **anxiety-like behavior**. Adult males failed to exhibit a location preference regardless of condition.

## 9. Gestational CBD increases time spent in open arms of an elevated plus maze.



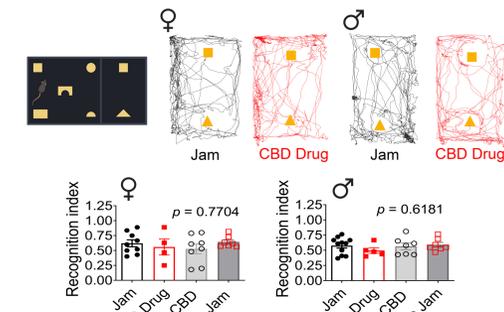
Gestational CBD exposure increased resident time in the open arms of an EPM for adult females, indicating gestational CBD exposure was anxiolytic.

## 10. Adult male mice exhibit reduced long-term memory if perinatally exposed to CBD.



Adult male mice exhibited reduced **long-term memory** if exposed to CBD during both gestation and lactation. There were no changes in **short-term memory**.

## 11. Perinatal CBD exposure has no effect on offspring performance in an attention task.



Perinatal CBD exposure had no significant effect on offspring performance in an object attention test, indicating no change in **ADHD-like behavior**.

## CONCLUSION

Mice can be acclimated to eating a 100 mg/kg dose of CBD in strawberry jam to provide an effective oral delivery for pregnant mice. CBD **does not alter maternal behavior** as assessed in a pup retrieval test. Oral consumption **does not change maternal weight gain, litter size, or gestation duration**, but it **decreases pup survival** to weaning age. Perinatal CBD exposure **modifies ingestive behavior and fuel utilization** in adult offspring. Adult mice exhibit **obsessive compulsive behavior** if exposed to CBD *in utero*. This behavior is mitigated in females if they are cross fostered to a drug free dam. Adult female mice have **reduced anxiety** as determined by the light-dark box and spend less time in the closed arms of an elevated plus maze. Adult male mice exposed to CBD *in utero* exhibit **reduced long-term memory**, which can be helped by cross-fostering. Finally, perinatal CBD exposure showed no effect on short-term memory or ADHD-like behavior.

## Funding

This work was funded by the **Florida Consortium for Medical Marijuana (MMJ)** Clinical Outcomes Research, FSU Bess Ward HITM Scholarship, and NIH T32000044. The authors declare no conflict of interest.