# Therapeutic Potential of Cannabidiol and WIN 55, 212-2-mesylate for Treatment of Anxiety

C. Huffstetler[1], B. Cochran[1], C. May[1], C. Silver[1], N. Maykut[1], C. Cedeno[1], D. Landi Conde[1,2], and D.A. Fadool[1,2,3]

The Florida State University, [1]Department of Biological Science, Tallahassee, FL-32306;
The Florida State University, [2]Program in Neuroscience, and [3]Institute of Molecular Biophysics, Tallahassee, FL-32306

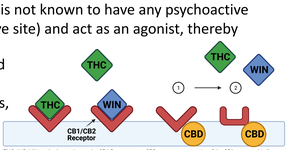
program in NEUROSCIENCE

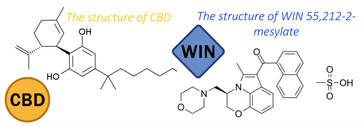
FLORIDA STATE UNIVERSITY

## Introduction

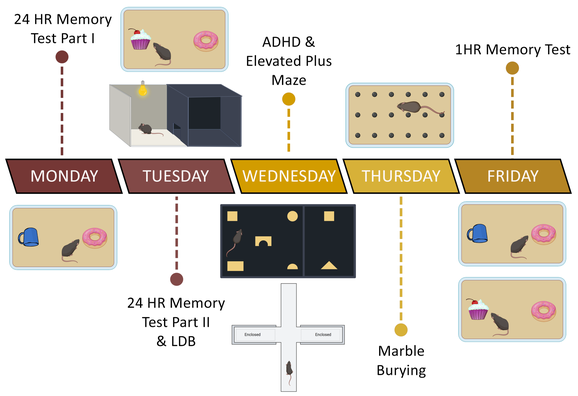
*Cannabis sativa* contains more than 100 phytocannabinoids, the most popular being Δ9-tetrahydrocannabinol (Δ9-THC), the psychoactive component of marijuana, and **cannabidiol** (CBD), the major non-psychoactive component [3]. As the medicalization of marijuana occurs across the United States, the psychoactive component of the cannabis plant, Delta9-**Tetrahydrocannabinol** (THC), has gained recognition for its therapeutic potential. Meanwhile, its non-psychoactive counterpart, cannabidiol (CBD), has been marketed to treat Parkinson's disease, Chron's disease, dystonia, attention-deficit hyperactivity disorder (ADHD), inflammation, depression, fibromyalgia, epilepsy, and most commonly, anxiety. Phytocannabinoids, which are cannabinoids synthesized from plants, bind to cannabinoid receptors to send signals throughout the body to maintain proper homeostasis. To understand how these cannabinoids act on the body, a general understanding of cannabinoid receptors is necessary. The two main receptors that are pertinent to this project are CB1 and CB2, found mainly in the brain and peripheral organs, respectively [2]. The CB1 receptor is mainly responsible for inhibiting the release of neurotransmitters in the neurons in which it is present [5]. Thus, activation of CB1 will decrease either the inhibitory or excitatory drive of the neuron, resulting in physiological changes involved in regulating homeostasis (depending on the presynaptic terminals in which the receptor is located) [4]. In our study, CBD will bind to the side of the CB1 receptor and act as a non-competitive antagonist; it will prevent other molecules from activating the receptor (also called allosteric inhibition). Similarly to THC, **WIN 55, 212-2-mesylate** (WIN) is an agonist for the cannabinoid receptors CB1 and CB2, but it is not known to have any psychoactive properties. It binds to CB1's pocket (called its active site) and act as an agonist, thereby activating the receptor.


Given the over the counter accessibility of CBD and the fact that anxiety disorders are the most common type of mental illness in the United States, our objective was to access the therapeutic potential of cannabinoid receptor agonists and antagonists for treatment of anxiety and attention deficit. We hypothesized that CBD might be anxiolytic when acutely or chronically administered via IP injection to a newly found mouse model of anxiety and attention deficit (Kv1.3-/- mice). Kv1.3 -/- mice lack potassium (Kv) channels in cells that can be found in the olfactory bulb, hippocampus and the pyriform cortex (PC). This causes a decreased repolarization, resulting in hyperexcitability, and usually, heightened anxiety. We analyzed the effects of chronic vs. acute administration of CBD and WIN, as well as the concentration of drug administered (dose responsivity).



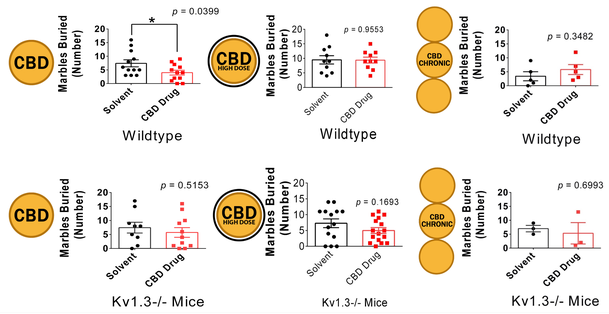

## Methods




All mice used in this study were housed in *vivaria* with a reverse 12/12-hour light/dark cycle (lights off at 8:00 A.M. and on at 8:00 P.M.). Experiments were approved under protocol number #202000036 by the Florida State University Institutional Animal Care and Use Committee (IACUC). Experiments were performed on approximately 3-month-old male and female C57BL6/J mice **(WT and Kv1.3−/−)**. IP injections were administered 30 minutes prior to testing.

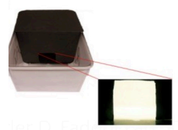
## 1. IP administration of low dose CBD acutely causes a reduction in obsessive compulsive behavior, but is ineffective at a high dose or with chronic administration, whereas Kv1.3-/- mice do not respond to CBD regardless of concentration or duration of administration.

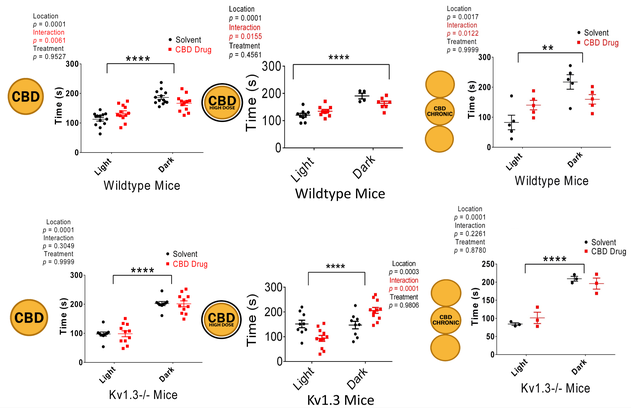
### Marble Burying Test




## 2. Mice of both genotypes prefer the dark compartment (state anxiety). IP administration acutely of either dose, or chronic administration, reduces anxiety in WT mice. Low dose or chronic administration of CBD in Kv1.3-/- is ineffective, but high dose is anxiogenic.

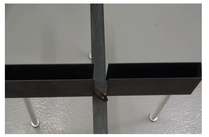
### Light-Dark Box

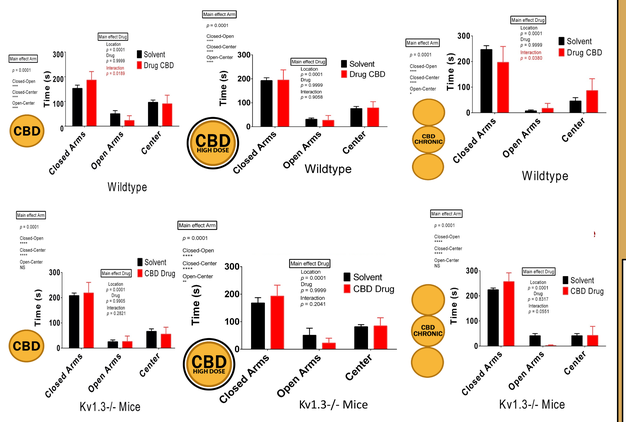




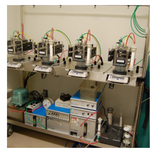

## 3. IP administration acutely of low dose CBD was anxiogenic to WT mice in the EPM but became anxiolytic after chronic administration. Kv1.3-/- had no change in EPM behavior, regardless of dose or duration of CBD.

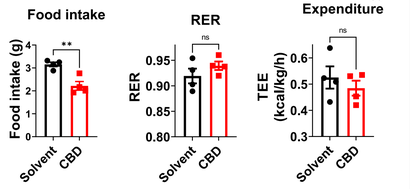
### Elevated Plus Maze



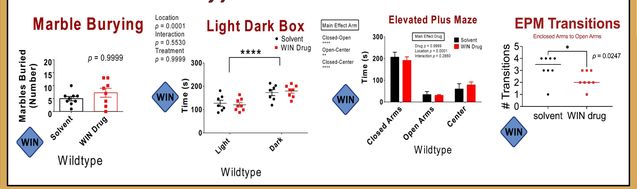



## 4. Chronic IP administration of CBD in WT mice decreases food intake but does not alter RER or total energy expenditure

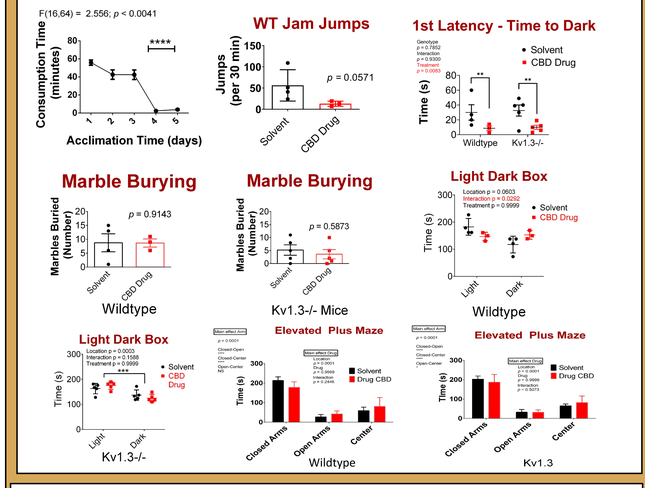





## 5. Acute treatment with WIN largely had no effect on obsessive compulsive or anxiety-like behaviors, but did decrease open arm transitions (aligned with enhanced anxiety).




## 6. Strawberry jam can be used as a vehicle for oral administration of CBD. Oral administration of CBD decreases time to the light compartment.




## Conclusions

1. Acutely administered CBD reduces obsessive compulsive and anxiety-like behaviors in wildtype mice as shown in marble burying tests, but the effect is not lasting with chronic administration.
2. Both genotypes of mice prefer dark compartments, therefore, have some state anxiety, however, CBD is effective both acutely and chronically in relieving anxiety of the light compartment in wildtype, but not Kv1.3-/- mice. At high dose, CBD is anxiogenic for Kv1.3-/- mice.
3. Length of CBD treatment affects extreme anxiety in mice using the elevated plus maze. Acute CBD demonstrates enhanced anxiety, whereas chronic CBD was effective in reducing anxiety-like behavior.
4. Acute treatment of wildtype mice with WIN showed no significant effect on obsessive compulsive or anxiety-like behaviors, but did decrease open arm transitions.
5. Chronic CBD treatment in wildtype mice can reduce food intake without changes in RER or total energy expenditure.
6. Mice of both genotypes were examined for object memory and attention changes (ADHD) in response to acute and chronic CBD. IP administration of drug had no significant effect on these cognitive behaviors.

## Future Considerations

Because CBD can be administered in different forms, we plan to test several other forms of delivery (oral, vapor and intranasal). We have begun oral delivery approaches. We also plan to separate our data by sex. We will also complete high concentration of WIN (dose responsivity).

## References

[1] Huang et al. 2018. Elevated anxiety and impaired attention in Kv1.3 knockout mice. *Frontiers Beh Neurosci* 12:49.

[2] Zou S, Kumar U. 2018. Cannabinoid Receptors and the Endocannabinoid System: Signaling and Function in the Central Nervous System. *Int J Mol Sci* 19(3):833.

[3] Mackie K. 2008. Cannabinoid receptors: where they are and what they do. *J Neuroendocrinol.* May;20 Suppl 1:10-4.

[4] Pertwee RG. The pharmacology of cannabinoid receptors and their ligands: an overview. 2006 *Int J Obes (Lond).* 30 Suppl 1:S13-8.

[5] Bear MF, Connors BW, and Paradiso MA. 2016. Neuroscience: Exploring the Brain, Fourth edition. 60-62.

## Funding

This work was funded by the Florida Consortium for Medical Marijuana (MMJ) Clinical Outcomes Research. The authors declare no conflict of interest.

Consortium for Medical Marijuana Clinical Outcomes Research